TRANSIENT AND OSCILLATORY BIOELECTRIC POTENTIALS AND THEIR RELATION TO AUTOMATIC CONTROL MECHANISMS IN PLANT ROOTS

by

IVAN S. JENKINSON, B.Sc. (Queensland)

Thesis submitted for examination for the degree of

DOCTOR OF PHILOSOPHY

UNIVERSITY OF TASMANIA

HOBART

March, 1960.

PREFACE

The investigation described in this thesis was conducted between March 1957 and November 1959 at the Biophysics Laboratories in the Physics Department of the University of Tasmania, Hobart. This research was carried out during the tenure of a C.S.I.R.O. Studentship in Biophysics for which the author is grateful.

The author wishes to express his gratitude to Dr. B. I. H. Scott who supervised the research throughout. Dr. Scott originally developed the plant growth meter and the methods of observing the extracellular potentials produced by plant roots as described in Section I, 2. He was also the first to observe the bioelectric oscillations, of period 4 to 7 minutes, described in Section I, 3. This work has been reported in the following publications:

McAulay, A. L. and Scott, B. I. H. (1954) - A new approach to the study of electric fields produced by growing roots. Nature 174:924.

Scott, B. I. H., McAulay, A. L. and Pauline Jeyes (1955) - Correlation between the electric current generated by a bean root growing in water and the rate of elongation of the root. Aust. J. Biol. Sci. 8 : 36.

Scott, B. I. H. (1957) - Electric oscillations generated by a bean root growing in water and the rate of elongation of the root. Aust. J. Biol. Sci. 10 : 164.

The research described in Section VI, which is largely subsidiary to the main theme of this thesis, has been published as follows:

Jenkinson, I. S. (1958)- Transient bioelectric potentials produced by electrically stimulated bean roots. Aust. J. Biol. Sci. 4 : 485.

Papers are in preparation embodying the work presented in Sections II to V.

The author is indebted to Professor A. L. McAulay, Miss H. Gulline, Mr. G. Findlay, Mr. I. Newman of the Physics Department and to Dr. D. Paton, Dr. W. Jackson and Mr. D. Thomas of the Botany Department for much helpful discussions and constructive criticism.

The technical assistance of Mrs. A. Fulton, Miss J. Watchorn, Mr. D. LeSouef and Mr. D. Millwood of the Physics Department is very gratefully acknowledged.

This thesis was typed by Mrs. P. Top of the Physics Department for which the author is most grateful. The assistance of Miss J. Watchorn in preparing the diagrams and the efforts of the Photographic Section of the University of Tasmania in producing the photographic reproductions in this thesis is gratefully acknowledged.

Ivan S. Jenkinson,
University of Tasmania,
February, 1960.

CONTENTS

I

INTRODUCTORY SECTION: THE STEADY, TRANSIENT AND OSCILLATORY POTENTIALS OF BEAN ROOTS

I

THE STEADY, TRANSIENT AND OSCILLATORY POTENTIALS OF BEAN ROOTS

1. Introduction

In all dynamic systems, whether physical or biological, in which interaction of a large number of variables occurs, the study of transient and oscillatory phenomena has in general facilitated the elucidation of the complex processes involved in such systems. The study of the animal nervous system is a classical example of this. In general oscillatory and transient phenomena indicate some instability in the rather delicate balance between different variables in a physiological system.

The study of oscillations in the bioelectric potential pattern of bean roots has led to the conclusion that these are a symptom of instability in a negative feedback system of control acting between cartain physiological variables in the biological organ. From an investigation of this feedback oscillator considerable information about the interaction of these physiological variables has been revealed.

In this section it is proposed to describe briefly the bioelectric potentials around a bean root immersed in a weakly conducting salt solution. These potentials, both steady and variable, have been described previously in publications from this

laboratory together with the measurement methods of both the potentials and the growth rate of plant roots. (McAulay and Scott 1954; Scott, McAulay and Pauline Jeyes 1955; Scott 1957; Jenkinson 1958).

In succeeding sections the results of experiments designed to elucidate the mechanism of potential variations will be described. Special attention will be directed to the study of a particular type of spontaneous potential variation, namely sustained bio-electric oscillations. However it seems desirable to summarise first the basic phenomena of the steady and variable potentials exhibited by bean roots.

2. Experimental Material and Methods

The material used was a Long Pod variety of the broad bean Vicia faba L., which was grown in continuously circulated and aerated tap-water at 25°C. Plants 2-3 days old with roots about 4 cm. long were used in most experiments.

The plant under investigation P was mounted vertically in the measuring tank with the root, but not the cotyledons, immersed in a bathing solution of 10^{-4}M KCl (Figure 1). The bathing medium was continuously replenished and circulated by fresh aerated solution. The temperature of the bathing solution was

thermostatically maintained at 25 ± 0.5°C.

Bioelectric potentials were measured by means of probes consisting of lengths of transparent "Nylex" tubing N (2mm bore) the ends of which were placed close to the surface of the root at various points along its immersed length (Figure 1). The other end of each tube dipped into an insulated plastic cup C, the cups and tubes being filled with the same solution as that of the bath. Tubes came from five points near the plant while a sixth came from a distant point in the bath thus acting as a reference zero of potential. Each of these cups was connected in turn to the measuring circuit by means of a liquid-switching arrangement. Associated with each cup was a glass bridging tube B (also filled with the KCl bathing solution) which could dip into C and make a connection toa small bath R, insulated from the main bath. The bridging-tube made this connection when the solenoid S, on which it was mounted, was energised. One of the calomel half cells E dipped into the bath R, the other into the main bath at a point distant from the plant. These were connected to an electrometer, the output of which was fed to a Cambridge six channel quick-acting recorder with a maximum full-scale deflection of 1 m.V.

This recorder produced dots of different colours to distinguish

FIGURE 1

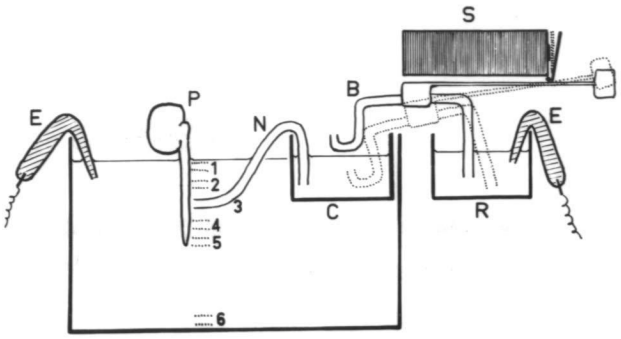
Schematic diagram of the measuring bath to illustrate the liquid switching arrangement. For simplicity only one of the liquid-conducting paths from the plant to the rear bath is shown (From Scott 1957).

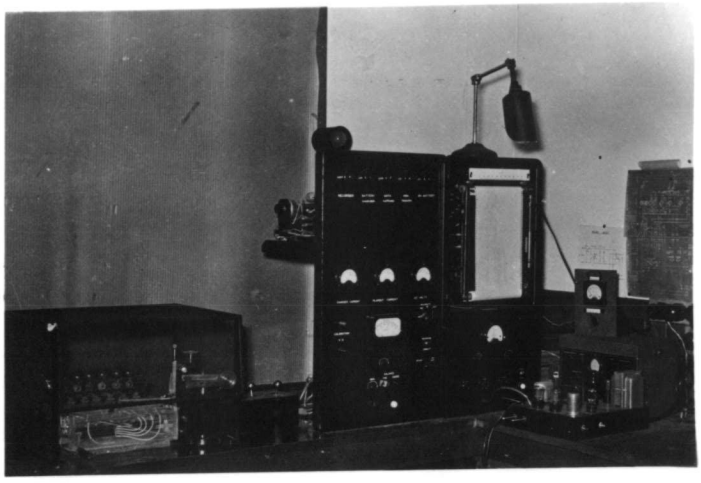
FIGURE 2

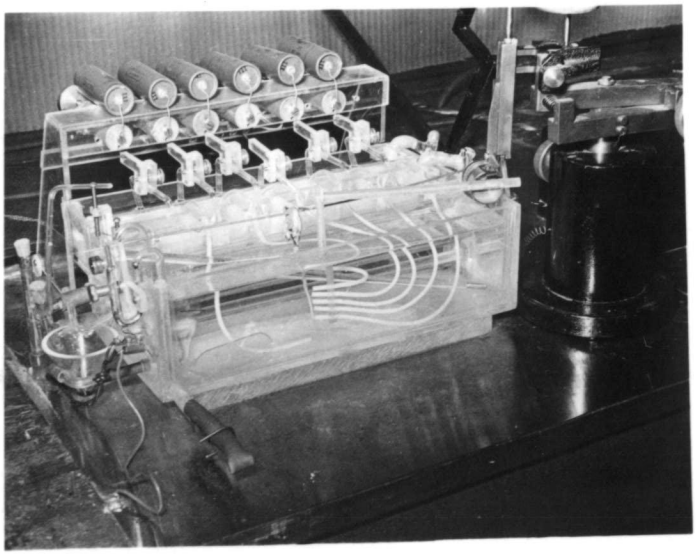
Apparatus for making automatic records of plant root potentials (From Scott 1957).

FIGURE 3

Measuring bath showing a bean root set upfor measurement. (From Scott 1957).


S
B
E
P
N
E
1
2
3
C
R
4
5
6





the different channels, the time between successive dots being 5 seconds so that each channel was recorded at half-minute intervals. This was synchronised with the energising of the liquid bridge solenoids so that potentials at five points along the plant root and one at a distant indifferent point were measured at the half minute intervals. For most experiments the chart was driven at three inches per hour. Figure 2 shows a photograph of the whole apparatus whilst in Figure 3 a close-up is shown of a plant set up for measurement.

Figure 4 shows the electrometer circuit using a pair of matched ME 1400 valves in a balanced circuit designed to be as insensitive as possible to changes in the high and low tension supplies. Currents flowing in the input of this stage were never greater than 10^{-12} amperes under measurement conditions.

Growth rate measurements were made with the equipment shown in the photograph of Figure 5. The principle is indicated diagramatically in Figure 6. Potassium chloride solution (10^{-4}M) from a constant head of about 120 cm of water flowed along a tube in which there were two constrictions X and Y which restricted the flow of water by about the same amount. The constriction at Y was variable, being covered by a flexible flap against which the root tip pressed lightly.

FIGURE 4

Circuit diagram of the electrometer stage used in the automatic recording apparatus. (From Scott, Ph.D. thesis, 1956).

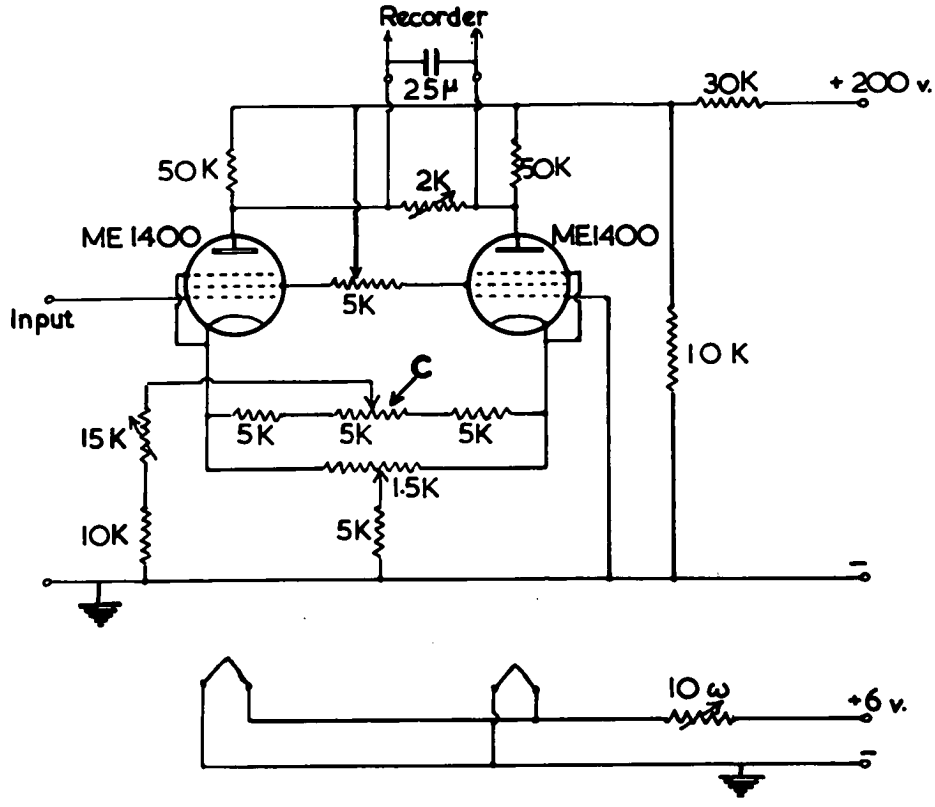
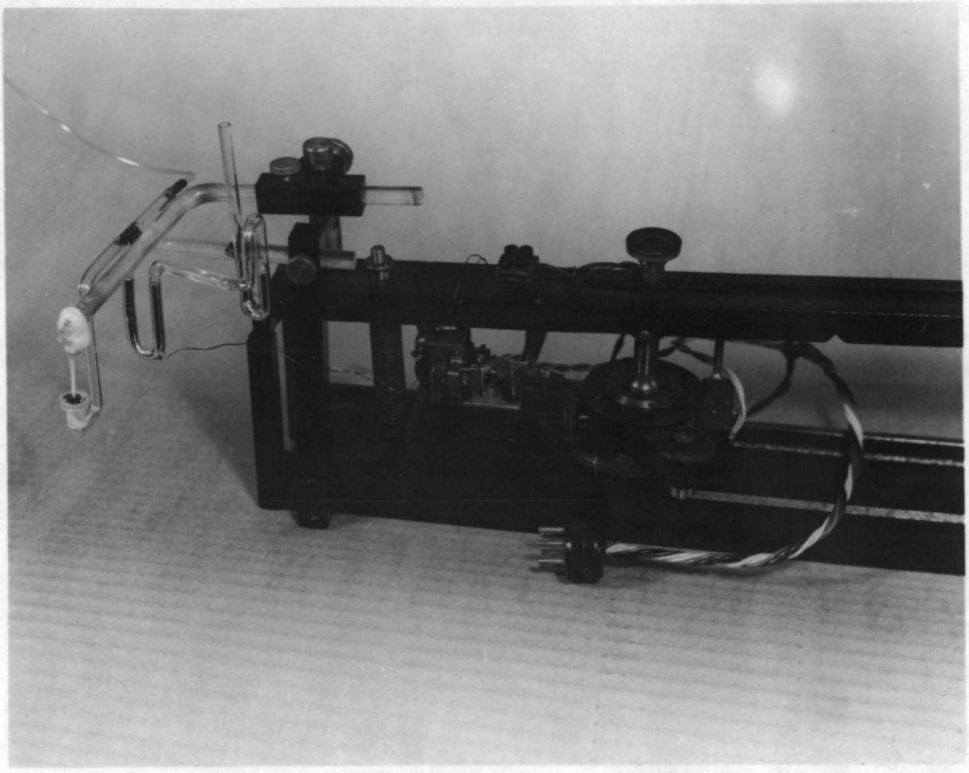

Recorder
25μ
30K
+200 v.
50K
2K
50K
ME 1400
ME1400
Input
5K
10 K
C
15 K
5K
5K
5K
1.5K
10K
5K
10 ω
+6 v.

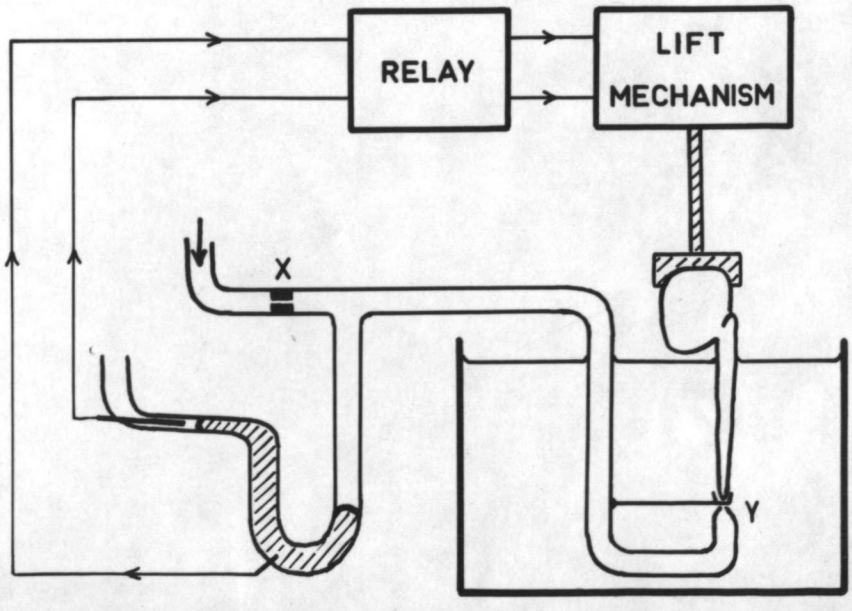
FIGURE 5

Apparatus for measuring the elongation rate of plant roots. (From Scott, Ph.D. thesis, 1956).

FIGURE 6

Schematic diagram illustrating the principle of the growth-rate meter. (From Scott 1957).




RELAY
LIFT
MECHANISM
X
Y

The method utilised the fact that a small increase in the resistance to the flow of water at Y caused a considerable increase in the water pressure between X and Y. This increase caused electric contacts in the mercury manometer to close, thus actuating the lift mechanism. This raised the plant through an increment of 1μ thereby reducing the pressure on the flap and breaking the electric contact in the manometer. Thus the growing plant was raised in 1μ steps each time the manometer contacts closed.

The lift mechanism employed a screw and ratchet of a Cambridge rocking-arm microtome which was operated by a solenoid. The solenoid was energised by an electric pulse from a relay circuit each time the manometer contacts closed. If a single 1μ elevation of the plant was insufficient to break the contacts (eg. if the root grew several microns suddenly) it was arranged that pulses from the relay continued to energise the solenoid at 1 or 2 second intervals until the contact broke.

The number of growth increments of 1μ occurring each minute was recorded on the same chart as the potential measurements. Each pulse from the relay turned the movable contact of a variable resistance through a small constant angle. A constant current was passed through this resistance, consequently the potential across it was proportional to the number of growth increments.

This potential was registered by the recorder each minute, one of its channels being used for this purpose. The integrator then automatically reset to zero and began counting the increments during the next minute.

3. The Steady and Oscillatory Potential Pattern Around a Bean Root

Figure 7 shows the general pattern of potentials along a plant root immersed in a solution of 10^{-4}M KCl. For this plant the root was immersed by different amounts. It is apparent that the potential pattern is similar in form for each case but it is expanded or contracted depending on the length of root immersed. In all cases the more mature regions of the root are electropositive with respect to the less mature parts. Moreover the total potential along the root increases with the length of root immersed.

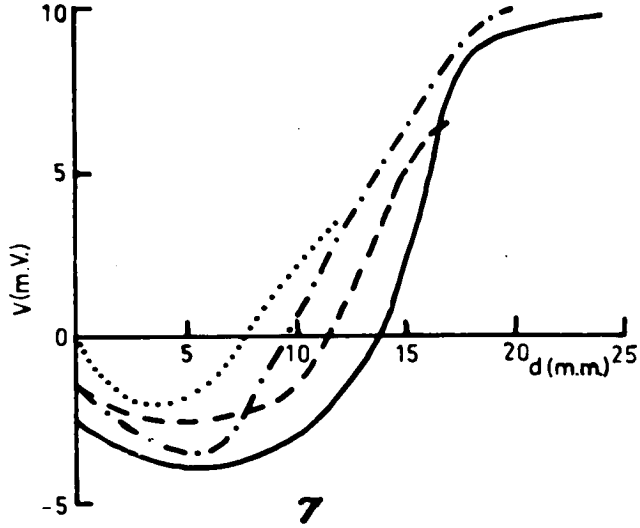
Scott, McAulay and Pauline Jeyes (1955) have shown that the potentials adjacent to points along the root are produced by the continuous flow of bioelectric current flowing out from the more mature regions of the root, through the bathing solution and returning to the plant at the less mature root tissue nearer the tip. The ohmic drop in potential along the current circuits in the bathing solution constitutes the measured bioelectric potential. Hence as the length of root immersed is increased, the total amount

FIGURE 7

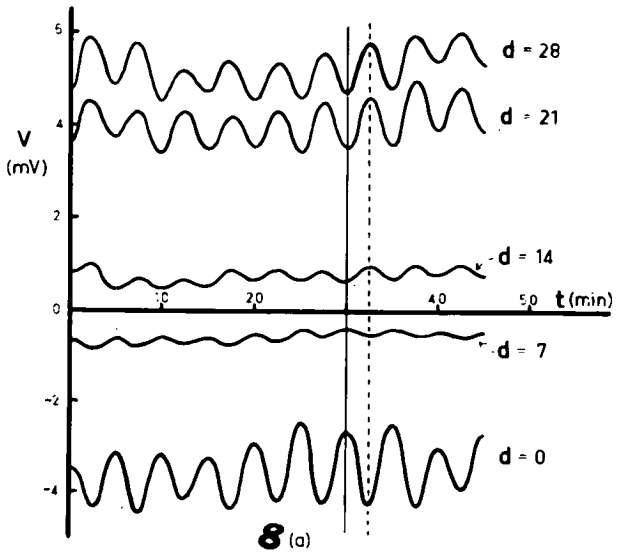
Relation between the root potential (v) and the distance from the root tip (d) for a plant immersed by different amounts as indicated on the graphs.

FIGURE 8

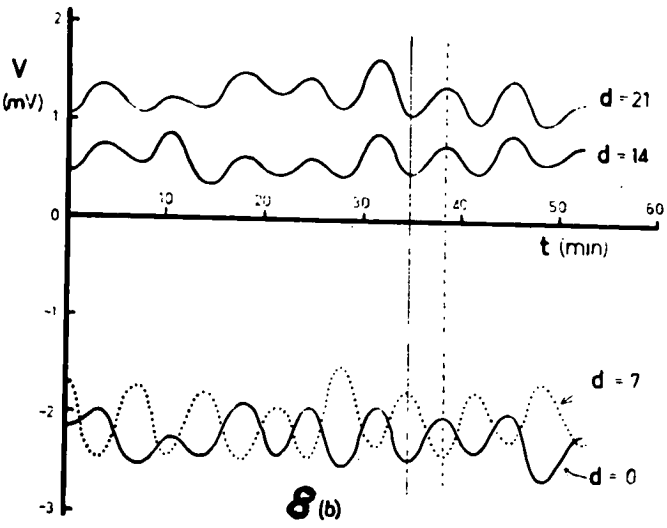
Time courses of spontaneous extracellular potential oscillations produced by bean roots. The distances (d) along the root from the tip where the potentials were recorded, are shown. In (a) there is only one phase reversal along the root while in (b) there are two.


10
5
0
-5
V(m.V.)
5
10
15
20
d (m.m)
25

7


6
4
2
0
-2
-4
V
(mV)
d = 28
d = 21
d = 14
d = 7
d = 0
20
40
50
t (min)

8 (a)


2
1
0
-1
-2
-3
V
(mV)
d = 21
d = 14
d = 7
d = 0
10
20
30
40
50
60
t (min)

8 (b)

of bioelectric current increases also, thus increasing the total potential along the root.

The potentials observed at different points along the root occasionally exhibit regular variations in the form of sinusoidal oscillations. These spontaneous oscillations are superimposed on the steady potential pattern of the root. Figures 8(a) and (b) show typical examples of these sustained potential oscillations at various points along the length of the plant. The amplitude and phase of the oscillations varies with position along the length of the root. The most active region is nearly always within 8 mm. of the root tip, that is in the regions of the root containing the meristematic and elongating tissue. In Figure 8(a) the oscillations at the tip are in antiphase with those nearer the base of the root whilst in Figure 8(b) the oscillations 7 mm. from the tip, besides being largest in amplitude, are also in antiphase with the oscillations at the tip and at the more basal regions.

Such phase reversals are to be expected since a rise in potential of the water near one part of the root must be accompanied by a fall somewhere else along the root, if the electric current leaving the root is always to balance exactly the current entering it. As large amptitude oscillations are often observed near only a relatively small region of the root it appears that the

active source of the bioelectric oscillations is confined to this region, other parts of the root being largely inactive and exhibiting smaller oscillations merely because of the return path of the oscillatory current through them.

4. The Transient Potentials of Bean Roots

For plants which show no spontaneous oscillatory potential activity, damped potential oscillations can frequently be evoked by stimulation. Following such treatment as mechanical or electric stimulation, sudden changes in salt concentration of the bathing solution or exposure of the root to air for a short time, the potential pattern of the root is changed quite markedly. After the stimulus is removed, the plant's potential pattern recovers eventually to a steady state, not significantly different from that before stimulation, provided this is not too great. During this recovery time the transient potentials often exhibit overshoots and damped oscillations.

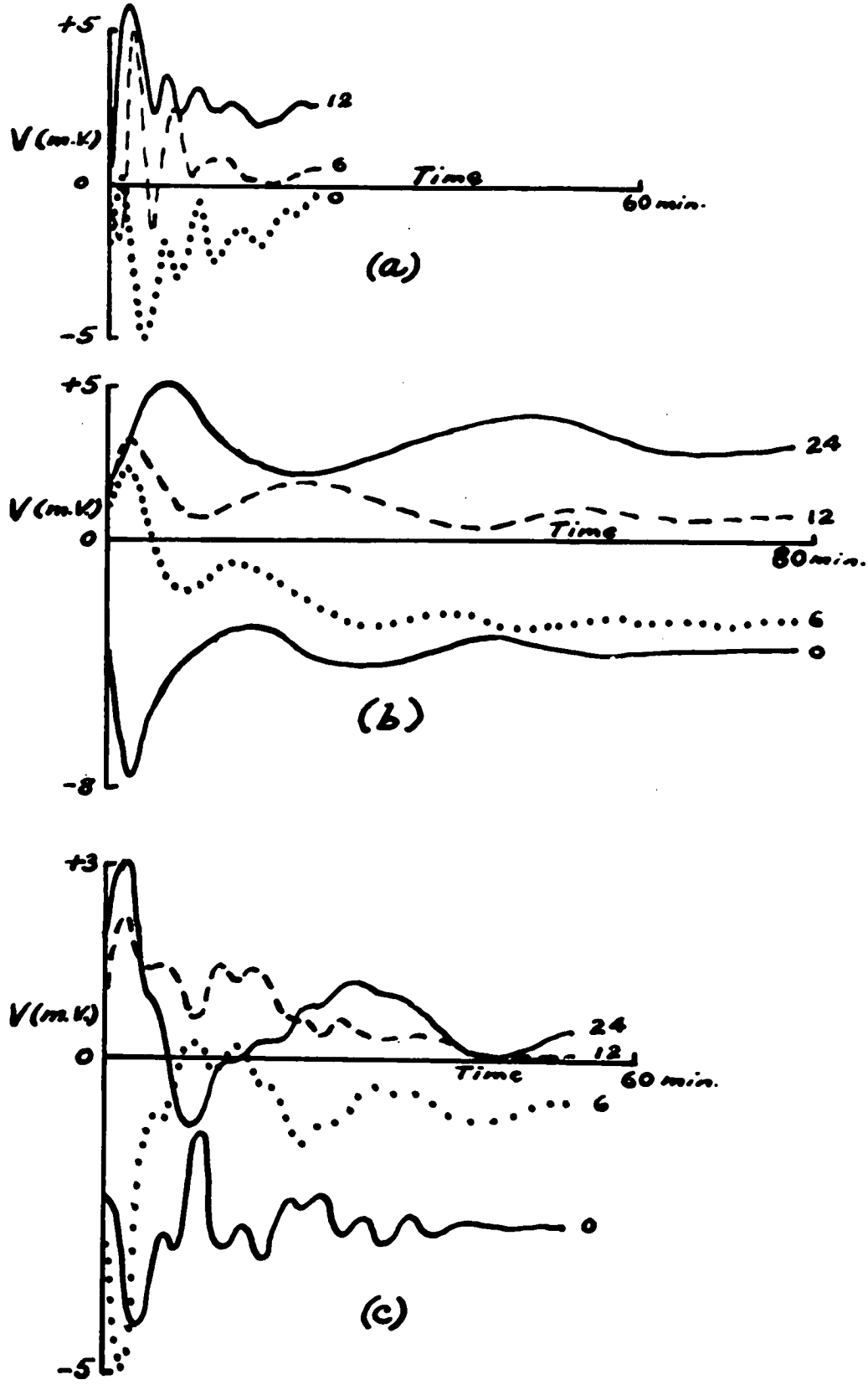
The potential oscillations appear to be of two types, frequently appearing together in the same transient (Jenkinson, 1958). One type is of approximately the same periodicity as the spontaneous potential oscillations viz. 4 to 7 minutes. An example is shown in Figure 9(a). In cases where spontaneous oscillations appear, the transient potential oscillations are of the same period.

FIGURE 9

(a) Transient oscillations of short period similar to that for spontaneous oscillations.

(b) Transient oscillations of longer period.

(c) Transient potentials showing both types of oscillations (a) and (b).


+5
V (m.V)
0
-5
12
6
0
Time
60 min.
(a)
+5
V (m.V)
0
-8
24
12
6
0
Time
60 min.
(b)
+3
V (m.V)
0
-5
24
12
6
0
Time
60 min.
(c)

Hence this type of transient oscillation may be identified with the spontaneous class of oscillations.

The other type of transient oscillation varies in periodicity from 10 to 100 minutes for different plants. In some cases the period varies from point to point along the length of the root. An example of this type of slow damped oscillation is shown in Figure 9(b). Frequently however the two types of oscillations occur together in transients. (Figure 9(c)). This suggests that the two oscillations are the result of different processes.

Although Scott (1957) described an oscillatory component in the rate of root elongation in some instances, these growth oscillations were not correlated with potential oscillations. These oscillations in growth rate did not occur at the same time as the potential oscillations and were moreover of considerably longer period (15 to 20 minutes) than the usual potential oscillations (4 to 7 minutes). In transient potentials, long period oscillations have frequently been observed but accompanying oscillations in growth rate have not appeared.

II

INTRACELLULAR PLANT ROOT POTENTIALS

II

INTRACELLULAR PLANT ROOT POTENTIALS

1. Introduction

The intracellular potentials of living tissue, unlike the extracellular potentials described in Section I, do not necessarily require the flow of bioelectric current through the tissue. Membrane potentials, such as those in nerve cells and large algal cells, result in part from diffusion and polarization processes which require no nett current transfer across the cell membranes. The Donnan system in cytoplasm also results in the appearance of electric potentials across the tissue. From a study of the intracellular potentials further knowledge of the electrophysiological processes contributing to the steady extracellular potentials and the production of potential oscillations should be gained. In addition it would be expected that such an investigation might reveal further information regarding the morphological position of the potential oscillator in the root.

Some large single plant cells such as the algae Nitella and Chara produce action potentials when stimulated (Osterhout 1931, 1934, 1936; Oda 1956; Findlay 1959). Oscillatory potential behaviour however is seldom observed in single plant cells whilst

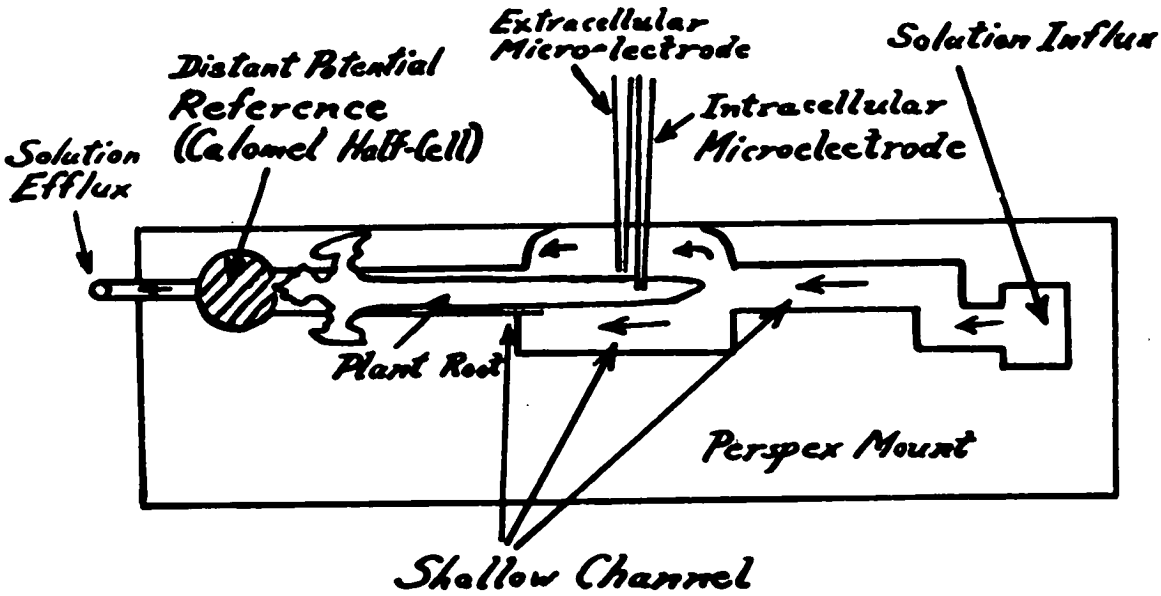
it can be quite marked in plant cell aggregates. This is similar to the situation in another class of tissue, namely neural tissue in which the characteristic response of single nerve cells is the impulsive action potential and not sustained potential oscillations. Aggregates of nerve cells, interconnected by synaptic junctions however, frequently exhibit spontaneous potential oscillations, the Alpha Rhythm of the brain being a typical example. It is considered that action potential responses in single nerve cells and spontaneous oscillatory potential behaviour in aggregates of neurones are intimately related phenomena (Eccles 1953). Hence the demonstration of action potentials and their possible transmission from one cell to another could be most important in elucidating the origin of potential oscillations in plants.

2. Experimental Methods

The plant under investigation was placed horizontally on a perspex mount with a suitable channel cut in it to allow the bathing solution to flow over and along the plant root (Figure 10). To facilitate the mounting of the root, most of the cotyledon material was usually removed, leaving only the plant root lying along the channel with the growing stem and the remainder of the cotyledons above water. This treatment was found not to affect

FIGURE 10

Experimental arrangement for measuring intracellular potentials showing the plant root, perspex mount, solution flow, distant reference electrode, extracellular and intracellular microelectrodes.


Extracellular
Micro-electrode
Solution Influx
Distant Potential
Reference
(Calomel Half-Cell)
Intracellular
Microelectrode
Solution
Efflux
Plant Root
Perspex Mount
Shallow Channel

either the extracellular or the intracellular potentials in any way.

The bathing solution flowed away from the plant via a distant reference electrode as shown in Figure 10. In this way the saturated KCl solution in the calomel half cell (the distant potential reference) was prevented from mixing with the required bathing solution applied to the plant. To ensure that the KCl solution in the calomel half cell was saturated, an excess of KCl crystals was maintained in it.

In some experiments the intracellular potentials were measured with respect to the distant indifferent reference electrode while in others, the potential was measured betweenthe microelectrode inserted in the tissue and another microelectrode placed close to the surface of the root but still in the bathing solution near where the probe insertion was made. In the latter, only the potential across the tissue or the transverse tissue potential was measured. The intracellular potential, measured with respect to a distant reference, is the sum of the transverse tissue potential and the extracellular potential at the point on the surface where the microelectrode was inserted.

The manner in which the plant was mounted for intracellular potential measurements was rather different from that used for purely extracellular measurements. In the former it was mounted

horizontally rather than vertically and part of the root rested against the perspex mount. However, it was found that the potential pattern along the root when mounted for intracellular recording is the same as that for purely extracellular measurements. Consequently it was decided that the results of intracellular and extracellular investigations are comparable.

Intracellular potentials were measured by means of microelectrodes made with pyrex glass tubing. After being immersed in boiling chromic acid for several hours and then washing with distilled water, the glass tubing was allowed to dry thoroughly. It was then drawn first in a gas flame and then on a de Fonbrune microforge. The tip diameters varied from 3μ to 10μ. Experiments carried out with microelectrodes thicker than 5μ were invariably checked using ones finer than 5μ. In general the results were found to be in agreement.

Potassium chloride solution was used to fill the microelectrodes, the concentrations used being 0.1, 0.3, 1 and 3 M. The results obtained at the different concentrations were found to be in agreement. Concentrations of 0.3 M and 1 M were used in most experiments.

The distant indifferent reference electrode (a calomel half cell) has already been described. The microelectrodes were connected

to calomel half cells also containing saturated KCl solution with excess crystals to ensure this.

The potential to be measured was fed to the input terminals of a Vibron electrometer, the output of which was either read directly from the meter on the front pannel of the instrument or fed to a single channel Evershed and Vignoles recording milliammeter. The full scale deflection of this recorder was 1 m.a. The response time of the Vibron electrometer was less than 0.1 second while that of the Evershed and Vignoles recording milliammeter was about 1 second. A chart speed of 6 inches per hour was used in most experiments.

In some experiments in which very rapid changes in potential were measured, another Evershed and Vignoles recorder, having a response time of less than 0.1 sec., was used. A chart speed of 0.5 inches per second was used for this recorder.

3. Injury Potentials and Spontaneous Intracellular Potentiation

As a microelectrode is being inserted in the plant root tissue it is possible to determine when the probe tip passes from one cell to the next because of the considerable mechanical force required to drive the tip through the tough cell walls. By referring to Figure 10 it is seen that the plant root is secured to the mount only at the basal end and is free to move, by bending,

along the rest of its length. As a probe is progressively inserted through the root tissue it is found that the probe tip enters the tissue in a succession of jumps, each jump occurring as the probe tip pierces a cell wall and so enters a new cell. In between these jumps the plant root bends slightly in the direction of the force applied by the probe tip being pushed against a cell wall before piercing it. Further, the potential recorded by the probe changes abruptly when each successive cell is entered. While the probe tip is inside a particular cell the potential is not dependent on the position of the probe tip within that cell. This is illustrated in Figure 11(a) which shows the variation of the intracellular potential recorded as a probe was driven radially through the root tissue at a constant rate. In this case the plant root was supported from the side opposite to that in which the probe was inserted. In this way no movement of the root occurred. It is seen that as the probe passes from one cell to another the potential decreases suddenly, then remains at a steady value for that cell. It is apparent however that the potential changes, which occur from cell to cell, decrease as the probe passes farther into the tissue until the potential eventually remains at a steady value for a large number of cells.

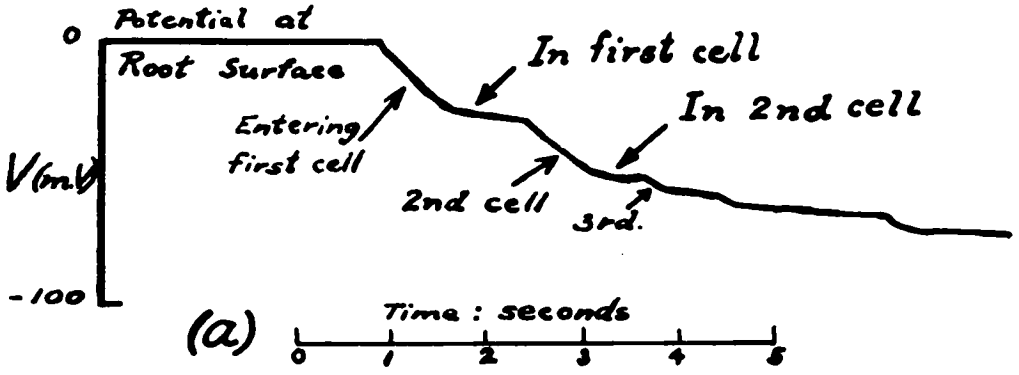
After a microelectrode has been inserted in the root tissue the recorded potential varies in a characteristic manner for a few

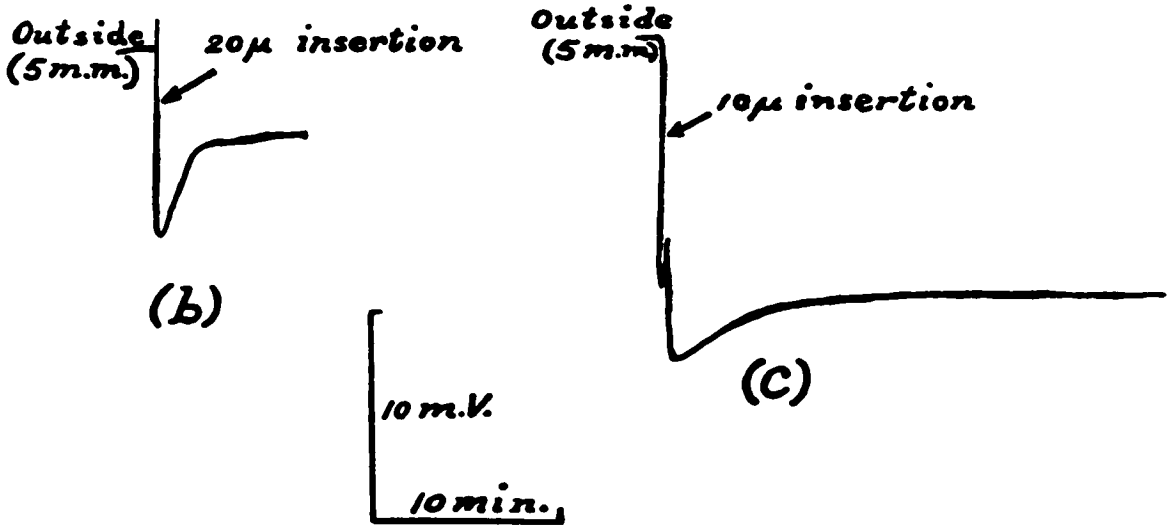
FIGURE 11

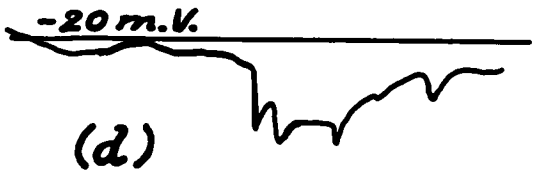
(a) Potential time course recorded as a probe was driven radially through the root tissue at a constant rate, the plant root being supported so that no bending occurred.

(b) and (c). The characteristic potential variations which occur when a probe is inserted in a plant root cell.

(d) spontaneous intracellular potential variations observed in epidermal cell.


0
Potential at
Root Surface
In first cell
In 2nd cell
Entering
first cell
2nd cell
3rd.
V(m.V)
-100
(a)
Time : seconds
0
1
2
3
4
5


Outside
(5m.m.)
20μ insertion
(b)
Outside
(5m.m.)
10μ insertion
(c)
10 m.V.
10 min.


-20 m.V.
(d)

minutes.

This is shown in Figure 11 (b) and (c). As the probe is inserted the potential suddenly becomes negative. Then after the insertion is completed the potential continues to decrease (i.e. becomes more negative) for about a minute until it reaches a maximum negative value. The magnitude of the potential then declines (i.e. the potential becomes less negative) for some five or ten minutes. It then attains a steady value which remains unaltered for several hours, except for slow fluctuation about the mean. The decline in magnitude of the potential from the maximum negative value soon after insertion is usually less than 50 percent of the potential of that cell with respect to its nearest outer neighbouring cell. This potential effect is essentially the same irrespective of whether the potential recorded is the transverse tissue potential or the intracellular potential with respect to a distant reference. This potential activity after probe insertion has not been found to depend on the concentration of KCl inside the probe nor on the diameter of the probe tip. This suggests that it is not caused by diffusion of KCl from the probe tip into the cell but merely results from the stimulus or injury occasioned by probe insertion. Consequently this potential variation could be termed an injury potential.

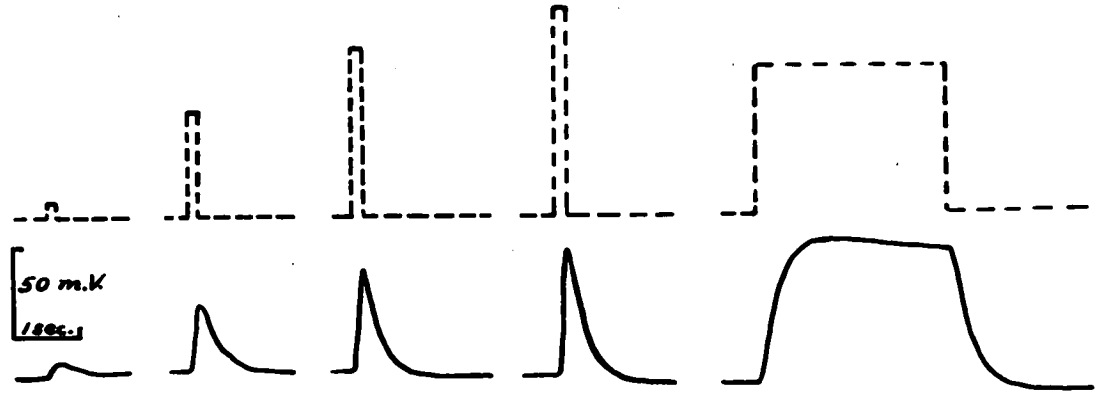
Occasionally spontaneous potential activity similar in form to the injury potentials is observed. This is shown in Figure 11(d). In this case it is seen repeatedly that the potential suddenly becomes more negative and then recovers at a slower rate, though the recovery may be only partial before the next negative-going spike occurs. This type of potential activity is similar to the inhibitory post-synaptic potentials in nerve cells. It is however quite different from the action potentials observed in large algal cells which resemble the excitatory potentials in nerve cells.

4. Stimulated Intracellular Potentiation

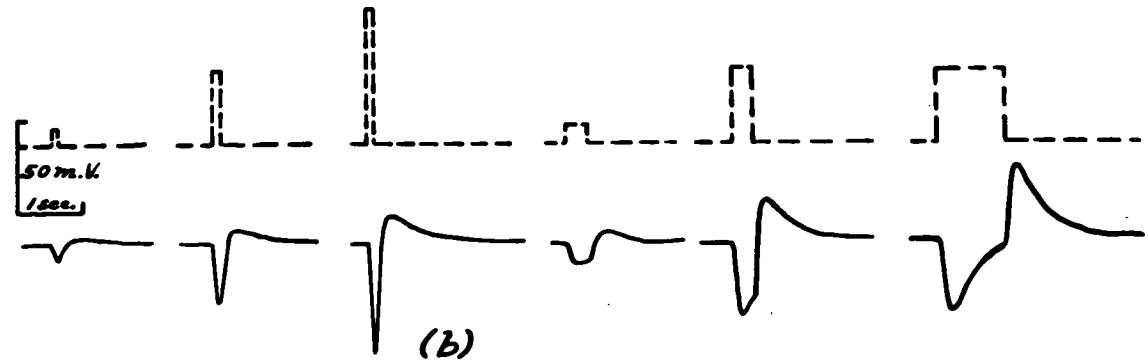
Although these spontaneous action potentials have been observed, attempts to evoke similar potentiation in response to depolarizing and hyperpolarizing current pulses have proved disappointing. Experiments were carried out in which a double-barrelled microelectrode was inserted in a cortical cell and stimulating pulses of depolarizing and hyperpolarizing current were applied from one side of the double microelectrode. The intracellular potentials recorded by the other side, with respect to a distant reference, are shown for a typical case in Figure 12(a). In this case depolarizing current pulses of different strengths and durations were applied successively. The relative current strengths and

FIGURE 12

Intracellular potential responses observed in a cortical cell when depolarizing current pulses of different strengths and durations (shown schematically by the dashed lines above the records) applied in (a) the cell where the potential was recorded and (b) in a cell about 1 mm. distant from the cell where the potential was recorded. Hyperpolarizing pulses evoke potential responses of the same form but of opposite sign.



(a)



(b)

durations are shown by the dotted lines on the record. After the pulse is applied, the intracellular potential gradually changes in the sense of the applied current pulse. If the current is applied sufficiently long, the intracellular potential reaches a new steady value dependent on the current strength applied. This is shown in the last of the series of Figure 12(a). When the current pulse is removed the potential begins to recover immediately and eventually returns in an exponential manner to its original steady value. Hyperpolarizing current pulses evoke potential responses of the same form but of the opposite sign. The potential response is not purely ohmic in that some integration effect occurs (capacitive effect) but there is no "action" component in the response. For all regions of the plant whether meristematic, elongating or mature, the response is the same.

When the stimulation probe is spatially separated from the recording probe a totally different response is observed. Figure 12(b) shows the intracellular responses of a cortical cell to depolarizing pulses applied in a cell about 1 mm. away. The response is the same in form but decreases in magnitude as the recording probe is moved further from the stimulator. When the pulse is applied, the potential changes in the opposite sense from that in Figure 12(a). The potential change reaches a maximum negative value and then begins to recover towards its former resting value even while the

current is still applied. When the current is removed the potential recovery proceeds at a faster rate but overshoots this value before eventually recovering completely. Current pulses applied in the opposite sense (i.e. hyperpolarizing) again evoke potential responses of the same form but of the opposite sign. The response is again the same in all parts of the root.

This response is obviously not ohmic in origin for the potential change is in the opposite sense from that of the applied current i.e. a depolarizing current pulse evokes a hyperpolarizing potential effect. It has the general features of a polarization effect and, although quite different from the responses in Figure 12(a), there are again no action potential features.

5. Steady Intracellular Potentials

The potential pattern inside plant roots has been determined by measuring the potentials inside successive cells as a probe is inserted first in the epidermis and then progressing transversely from cell to cell through the tissue. For different distances from the root tip, Table 1 shows the potentials of successive cells as a probe is passed transversely, beginning at the outside bathing solution (No. 0) into the first or epidermal cell (No. 1), then the second, or first cortex cell (No. 2) and so on. These

TABLE 1

STEADY INTRACELLULAR POTENTIALS RECORDED IN SUCCESSIVE ROOT CELLS

Cell No.	V (2mm)	V (3mm)	V (5mm)	V (10mm)	V (15mm)	V (20mm)
0	+ 4	-10	-10	+42	+17	+32
1	-17	-33	-20	-48	-17	- 5
2	-35	-41	-32	-62	-32	-31
3	-53	-54	-48	-71	-46	-44
4	-60	-56	-58	-73	-49	-52
5	-62	-57	-60	-80	-56	-52
6	-62	-57	-61	-80	-57	-58
7	-62	-57	-61	-80	-57	-58
8	-62	-57	-61	-80	-57	-58
9	-62	-57	-61	-80	-57	-58
10	-62	-57	-61	-80	-57	-58
11	-62	-57	-61	-80	-57	-58
Approximate Cell Thickness (Average)	30μ	32μ	35μ	45μ	50μ	50μ

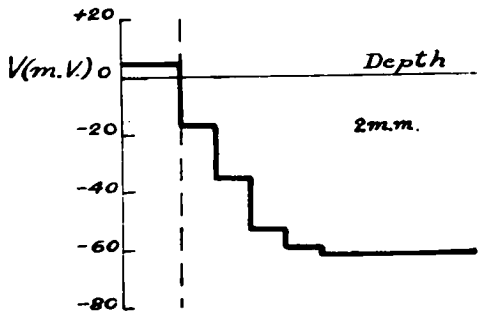
potentials were recorded with respect to a distant reference and were measured only after the potential of each cell had attained a steady value, i.e. after the cell had recovered from the injury caused by probe insertion. Although the cell thicknesses were not measured precisely, the average thickness taken for a number of successive cells was measured by the distance moved by the probe through the tissue.

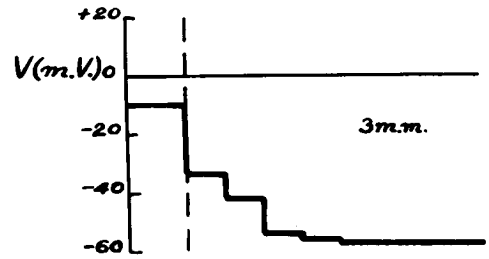
In Figure 13 the potential data obtained from the results tabulated in Table 1 are shown plotted against lateral distance through the root tissue. Again it is seen that the potential change from cell to cell is greatest at the surface of the root and diminishes progressively through the tissue, the potential eventually remaining at a constant value irrespective of the position inside the root tissue. Further it is apparent that the potential pattern is similar for all morphological regions of the plant root whether meristematic (2 mm), elongating (3 and 5 mm) or mature cells (10, 15 and 20 mm).

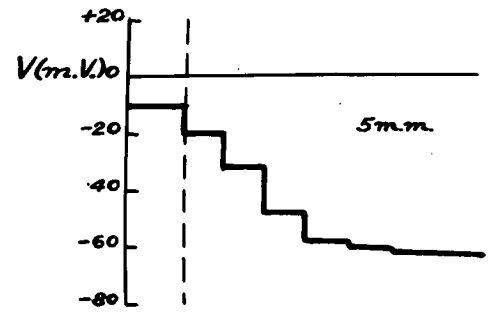
Since the intracellular potentials are negative at all regions of the plant root, they cannot be caused entirely by electric currents passing through the root tissue. For if this were so it would be necessary for a continuous current to pass from the bathing medium into all parts of the root which would result in

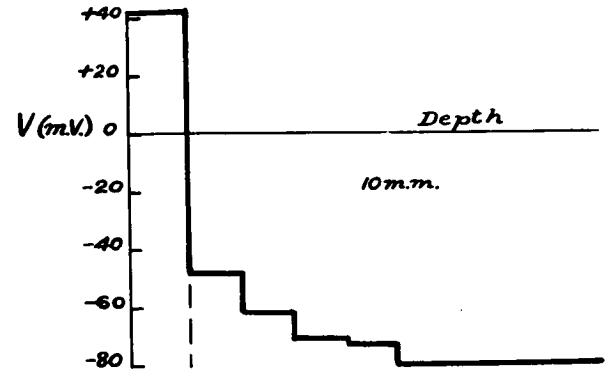
FIGURE 13

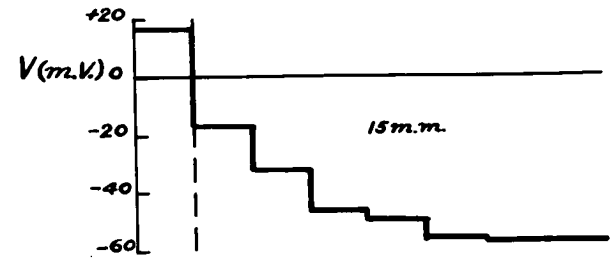
Steady potentials both extracellular (left of vertical dashed line) and intracellular (right) measured in successive cells with respect to a distant reference. These potentials are plotted against the depth in the tissue determined from average cell thicknesses in the root regions where the potentials were measured (see text and Table 1).

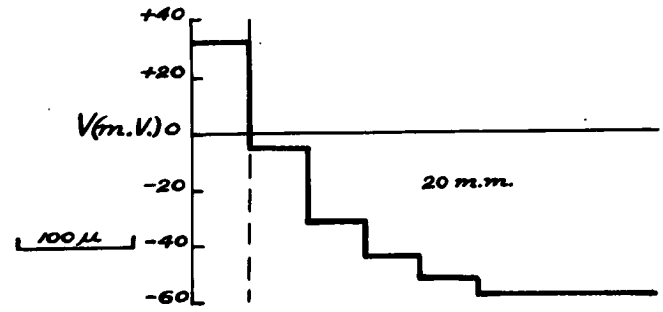

+20
V(m.V.) 0
Depth
2m.m.
-20
-40
-60
-80


+20
V(m.V.)0
3m.m.
-20
-40
-60


+20
V(m.V.)0
5m.m.
-20
40
-60
-80


+40
+20
V(m.V.) 0
Depth
10m.m.
-20
-40
-60
-80


+20
V(m.V.) 0
15m.m.
-20
-40
-60


+40
+20
V(m.V.)0
20 m.m.
-20
100μ
-40
-60

negative extracellular potentials at all regions round the plant root with respect to a distant indifferent reference electrode. This is known to be untrue, the bioelectric potentials observed in the plant's bathing medium being produced by currents passing out from one region of the plant and returning at another. Consequently the potential differences from cell to cell must be partly of a static type such as diffusion or Donnan potentials. There must of course be another potential component across the tissue caused by the bioelectric current which passes through it and out into the bathing solution.

6. Spontaneous Intracellular Potential Oscillations

In the comparatively infrequent instances of spontaneous oscillations in potential occurring in the bathing solution adjacent to a plant root, oscillations were also observed within the root tissue. An example of this is shown in Figure 14. In Figure 14(a) these oscillations were recorded outside and inside the tissue at various depths, using only one progressive insertion at 5 mm. from the root tip. It is seen that the maximum amplitude of the spontaneous oscillation occurs at a depth of 100μ. This is only a few cells deep and is just in the cortex. In Figure 14(b) the intracellular potentials at depths of 100μ were recorded at

FIGURE 14

(a) Oscillatory extracellular (top) and intracellular potentials recorded at various depths in the tissue, 5 mm. from the root tip.

(b) Oscillatory intracellular potentials recorded at various distances along the root from the tip, at depths of 100μ inside the tissue.

In both (a) and (b) the intracellular-potentials were recorded shortly after probe insertion.

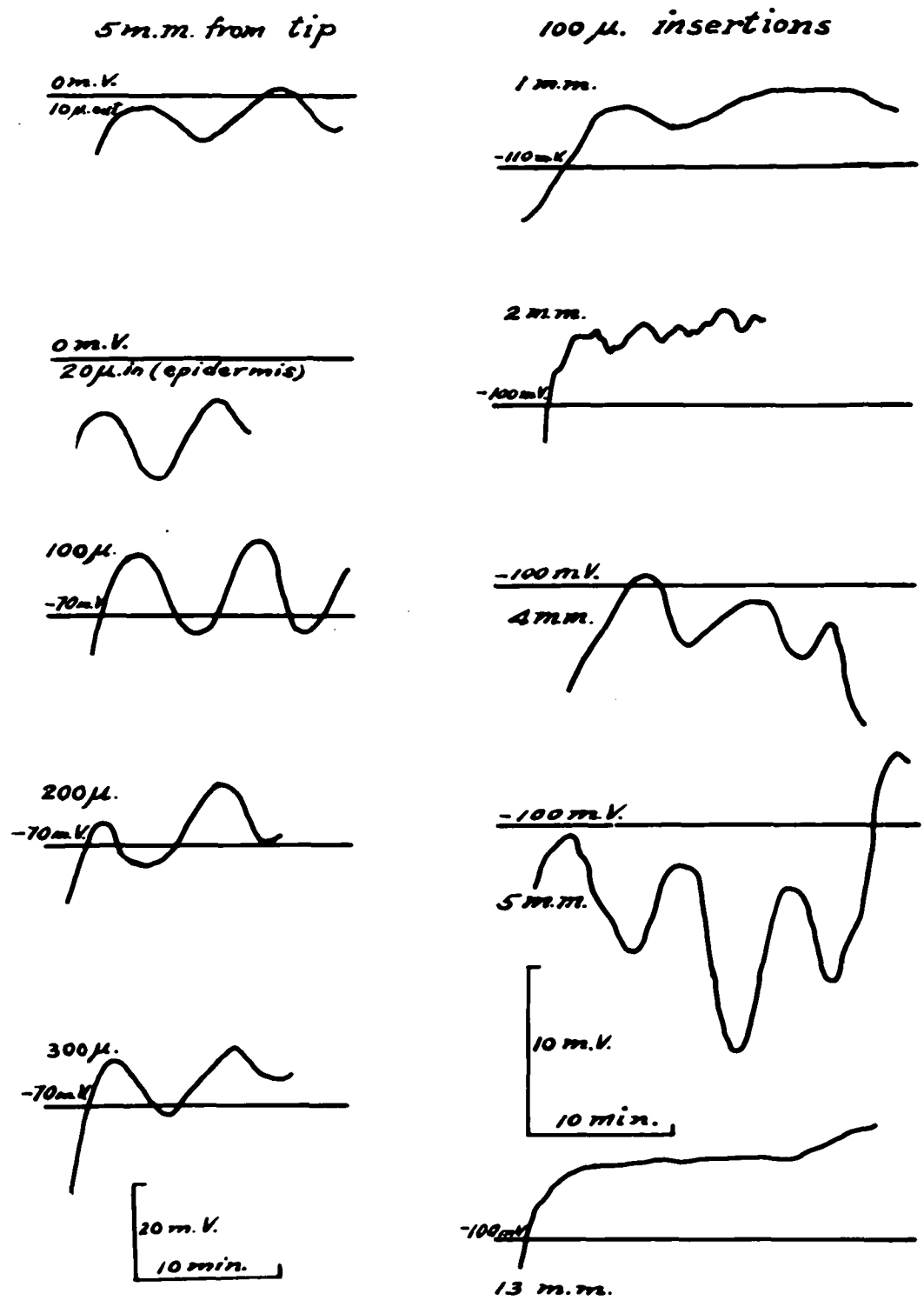
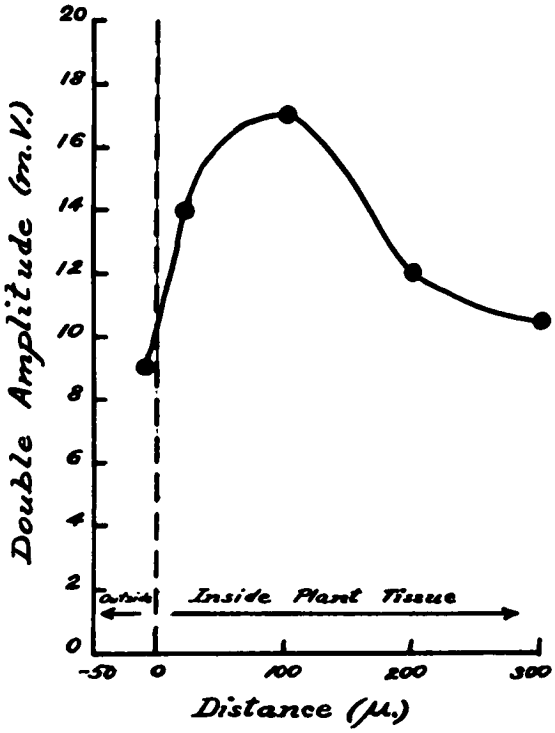

5 m.m. from tip
0 m.V.
10 μ. out
0 m.V.
20 μ. in (epidermis)
100 μ.
-70 m.V.
200 μ.
-70 m.V.
300 μ.
-70 m.V.
20 m.V.
10 min.
(a)
100 μ. insertions
1 m.m.
-110 m.V.
2 m.m.
-100 m.V.
-100 m.V.
4 m.m.
-100 m.V.
5 m.m.
10 m.V.
10 min.
-100 m.V.
13 m.m.
(b)

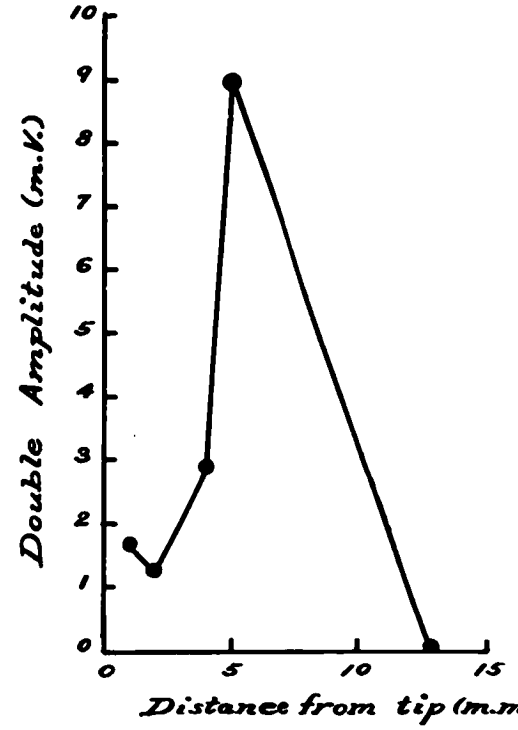
FIGURE 15

(a) Relation between double amplitude of spontaneous potential oscillations and depth in the root tissue, 5 mm. from the tip (data obtained from Figure 14(a)).

(b) Relation between double amplitude of spontaneous potential oscillations and distance along the root from the tip at depths of 100μ inside the root tissue (data obtained from Figure 14(b)).


Double Amplitude (m.V.)
20
18
16
14
12
10
8
6
4
2
0
Outside
Inside Plant Tissue
-50
0
100
200
300
Distance (μ.)

(a)


Double Amplitude (m.V.)
10
9
8
7
6
5
4
3
2
1
0
0
5
10
15
Distance from tip (m.m)

(b)

various positions along the root length. The oscillations of maximum amplitude occurred at 5 mm. from the root tip. Figures 15(a) and (b) show the data obtained from Figure 14 relating amplitude to position in the root. Again it is evident that the amplitude of the spontaneous oscillations is greatest at about 5 mm. from the tip and at a depth of about 100μ inside the tissue.

These results suggest that the bioelectric oscillator is morphologically situated in the region of immature cells which are actively elongating. Further, the maximum amplitude of the spontaneous oscillations occurs in the outer cortical cells where the steady potential differences from cell to cell are greatest except for the epidermis. Even in the elongating region the epidermal cells are relatively mature compared with those of the cortex. Hence it may be concluded that the cellular conditions associated with the bioelectric oscillator involve immature elongating tissue with relatively large potential changes from cell to cell.

III

INVESTIGATION OF THE FEEDBACK LOOP RESPONSIBLE FOR SPONTANEOUS OSCILLATIONS IN POTENTIAL

III

INVESTIGATION OF THE FEEDBACK LOOP RESPONSIBLE FOR SPONTANEOUS OSCILLATIONS IN POTENTIAL

1. Introduction

In biological systems, rhythmic and oscillatory phenomena, both damped and sustained, are frequently observed. Many of these, such as photosynthetic activity in plants, opening and closing of flowers, leaf movements, food consumption and sleeping habits of animals, are directly related to rhythmic or oscillatory variations in the environment, such as light and temperature, of the plant or animal.

Pittendrigh and Bruce (1957) have analysed in detail the eclosian rhythm of diurnal periodicity in Drosophila. Although they find that this rhythm has endogenous characteristics, it appears to be quite distinct from rhythms of much shorter periodicity totally unrelated to environmental variations. Such purely endogenous rhythms include the spiraling[1] of tendrils in climbing plants, the circulatory movements of roots, the oscillatory streaming and electric potentials of slime moulds and the oscillations and repetitive activity in the electric potentials of membranes and neurone aggregates in the animal nervous system. The oscillations in the potentials of plant roots come into this category.

Although it seems probable that the basic mechanism underlying many of the endogenous rhythms is the same in principle, the detailed physiological aspects are very different. It is proposed to describe some of the suggested mechanisms of various endogenous rhythms which appear to be physiologically unrelated but in which the same inherent principles are involved.

From voltage clamp experiments on the membrane of the squid giant axon Hodgkin and Huxley (1952) derived empirical expressions relating the potential to the sodium and potassium conductance of the membrane. These expressions predicted very accurately the observed action potentials and oscillatory behaviour of the membrane potential. However the fundamental process underlying the oscillations was ascribed to resistive, capacitive and inductive impedance properties of the membrane as suggested originally by Cole (1941). Arvanitaki (1943) and Sjodin and Mullins (1958) have also described sustained and damped potential oscillations as well as repetitive potentiation in the squid giant axon by means of an equivalent circuit containing resistance, capacitance and inductance.

Although the resistance and capacitance of the membrane are physically real, there seems no physical evidence of real inductance in the membrane. Hence although its electrical properties may be formally described by means of an inductance circuit this can only

be regarded as an equivalent circuit. However an alternative explanation has been suggested by Mueller (1958) who showed that many features of the excitatory potential activity of the single nodes of Ranvier can be simulated by a feedback control system involving time delays between the interaction of the potential, chemical equilibrium and the electromotance (i.e. depolarizing and repolarizing processes). By varying the parameters of the feedback system, many complex features of the potential variation can be explained. These features include the latency, refractory period, prolongation of the action potential in hypertonic solutions and potentiation under various conditions of externally imposed current, ionic and osmotic constitution. When the feedback system is unstable, rhythmic activity in the form of damped or sustained potential oscillations and repetitive activity is predicted.

Oscillations in the electric potential of neurone aggregates in the central nervous system and brain tissue are quite common e.g. the alpha rhythm in brain cortex. Eccles (1953) suggested that these oscillations could result from the interconnection of a large number of neurones forming a closed circuit which is self-energising. Potential activity in one neurone will eventually cause synaptic excitation of many other neurones in the circuit, there being time delays of about 0.5 milliseconds at the synapses.

(Coombs, Eccles and Fatt, 1955). This type of feedback system could well produce sustained potential oscillations, the periodicity depending on the synaptic delays and the interconnections of the neurones in the aggregate.

Adolph (1959) analysed the human antagonistic extensor-flexor system of the leg in terms of a multiple feedback loop with time delays in the interaction of nerve and muscular responses. The feedback system is shown to be stable except in certain pathological conditions when oscillatory behaviour, manifested as muscular twitch, results.

When a narrow beam of light of suitable constant intensity is focussed on the outer edge of the pupil of the eye, oscillations in pupil contraction occur. This oscillator has been treated as a feedback system controlling the intensity of light received at the retina. (Stark, Campbell and Atwood, 1958; Stark and Cornsweet, 1958). The feedback system variables appear to include the intensity of the incident light, the intensity at the retina and the muscular response controlling the pupil size.

Although the next system is purely physical it is very similar to biological membrane systems. In this model, Teorell (1959a) studied the behaviour of a charged artificial membrane separating two salt solutions of different concentration. The application of direct current across the membrane results in damped or sustained

oscillations in the potential and resistance of the membrane as well as the hydrostatic pressure and volumetric rate of solution transfer across the membrane. The mechanism to account for these phenomena involves the superposition of electrochemical and hydrostatic gradients across the membrane, resulting in variation of the membrane resistance caused by diffusion and electro-osmotic fluid streaming. A multiple loop feedback system links the membrane resistance, the potential, the hydrostatic pressure and the electrosmotic streaming (Teorell, 1959b). Kishimoto (1958) summarises the views of Frey-Wyssling (1949), 1953, 1958), Goldacre (1952), Goldacre and Lorch (1950), Seewy (1949, 1950 and 1952), and Seifriz (1942 and 1943) who attribute the oscillatory streaming of the slime mould Physarum polycephalum to a wave of contraction and expansion of contractile protein networks along a strand of the plasmodium. This rhythmic deformation of the contractile proteins or polyelectrolyte gels causes an oscillatory variation in their ionic concentration resulting in rhythmic potentials. By applying an oscillation in the hydrostatic pressure along a strand of the plasmodium, forced oscillations in the streaming and the electric potential are evoked. At the natural period (several minutes), resonance occurs and the amplitudes of both the streaming velocity and the potential are greatly enhanced.

The contractile protein molecules are thought of as elastic-inertia type oscillators which oscillate at some hundreds of cycles per second (the frequency of the wave). Phase lags are postulated between one protein oscillator and the next resulting in a wave motion travelling along the protoplasmic strand. Since the plasmodium is spatially bounded, a stationary wave system of to and fro streaming is set up. The interaction between the elastic protein oscillators along the strand and the motion of the whole protoplasm could be treated as a feedback system though this is probably not the most fundamental approach to the problem.

It is clear that feedback systems offer a very fruitful approach to the study of endogenous oscillations in a wide variety of biological systems. Scott (1957) suggested that the potential oscillations produced by bean roots could also be caused by a closed-loop feedback system of control acting between certain physiological variables. The suggested variables were the electric field, the auxin supply and the permeability of cell membranes. In this section experiments are described which were devised to test this hypothesis.

One of the standard methods of investigating a feedback system is to apply an external oscillation of varying frequency into one of the variables of the feedback loop. The resulting amplitude and

phase responses of all the variables then determine the properties of the loop. Alternatively if only one of the variables in the loop can be measured, oscillations in each of the variables may be applied externally, applying the oscillation to only one variable at a time.

As only bioelectric oscillations can be observed conveniently for the bean root system under investigation, the latter method of investigating the proposed feedback loop oscillator has been used.

Attempts were made to investigate the proposed feedback loop characteristics by applying an oscillatory electric field to the plant root. However, there were considerable technical difficulties involved in separating the applied oscillatory potentials and any bioelectric potentials which the plant might produce in response to the applied field. These technical difficulties limited the strength of the applied potential to about 0.1 V at which the current passed through the tissue was only of the order of 1 μ.a. For these low currents a method of measuring only the plant's bioelectric response was achieved. The response however was found to be negligible, probably because the applied currents were too small.

An indirect method of causing an oscillation in the bioelectric potential has now been developed. This is achieved by oscillating the osmotic pressure of the plant root's bathing solution. The oscillation in the bioelectric potential probably results from

an oscillation in the water content and salt concentration of the outer cells of the root.

Experiments have been carried out also in which an oscillatory concentration of the auxin, indole-acetic acid (I.A.A.), is supplied to the root's bathing solution. These oscillations in auxin concentration about the root have elicited oscillations in the bioelectric potential similar to those evoked by oscillations in osmotic pressure.

Another method of analysing a feedback system involves the application of a sudden change in one of its variables or an associated variable. The resulting transient behaviour in the other variables of the feedback loop yields a considerable amount of information regarding the type of interaction between the variables such as time delays and other functional relations. Some of the results obtained by this method will be described first.

2. Experimental Material and Methods

The plants were grown in continuously circulated and aerated tap water at 25°C. In most experiments the plants were removed from the culture bath and set up in the bathing solution of the measuring tank only about an hour before the commencement of experimental measurements. This time would probably not allow for

complete equilibration of the plant roots with the experimental bathing solution. However in some series of experiments the plants were allowed to equilibrate in the experimental bathing solution for fifteen hours or more before experimental measurements were begun. In most experiments 10^{-4}M KCl at 25°C was used as the bathing solution but in some cases 10^{-4} M $CaCl_2$ was used.

In order to produce an oscillation in osmotic pressure of the plant's bathing solution without changing the environment in any other way, a soluble, unionised substance, to which the plant membranes are practically impermeable, is required. The substance must be unionised, otherwise the conductivity of the plant's bathing solution and hence the bioelectric potentials would be affected. Since sucrose satisfies these requirements it has been used in most of the osmotic pressure experiments described in this paper. Although plant membranes have only a very low permeability to sucrose, it is however a physiologically active substance even in small concentrations once it enters a tissue (Brown and Sutcliffe, 1950). Consequently the osmotic pressure experiments were repeated with mannitol which has the advantage of being largely physiologically inactive as well as being almost as impermeable as sucrose. The results obtained with mannitol and with sucrose were in agreement.

The β Indole-acetic acid (I.A.A.) used in these experiments was weighed out 10 m.g. at a time and then dissolved in 10 c.c. of

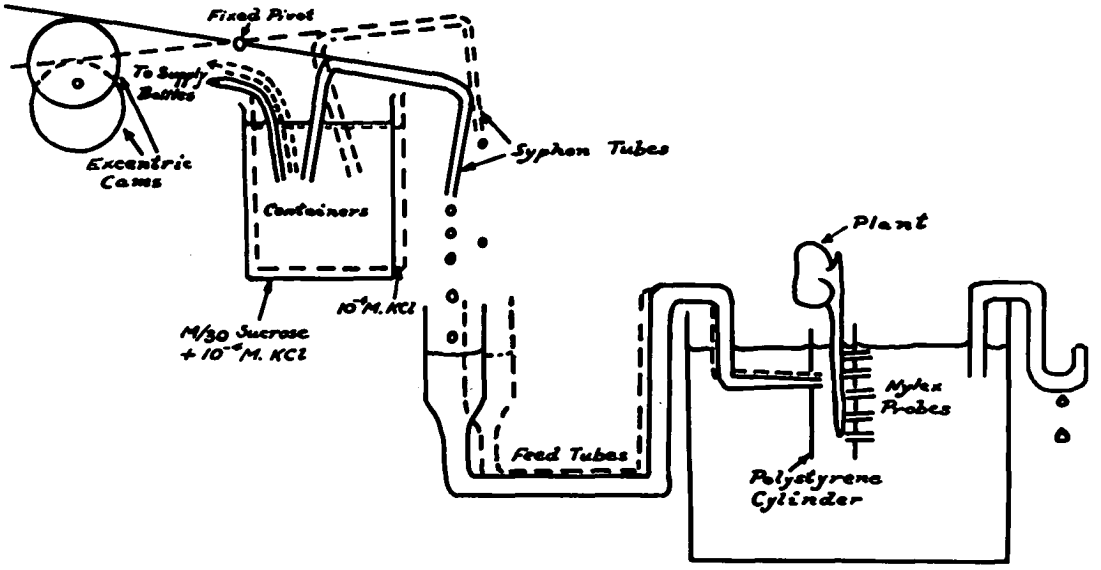
absolute alcohol. This was stored away from light and was made up with 10^{-4}M KCl to the required concentration on the day of use. While in use the stock bottle containing the I.A.A. in aqueous solution was shielded from light to help prevent deterioration. The concentrations of I.A.A. in aqueous solution used in these experiments were 10^{-9}, 10^{-7} and 10^{-5}M.

Figure 16 shows a diagram of the apparatus used to provide an oscillatory concentration of sucrose (or I.A.A.) in a solution of constant KCl concentration (10^{-4}M). Two constant-head supply bottles, one containing 10^{-4}M KCl and M/30 sucrose, and the other containing only 10^{-4}M KCl feed the two containers shown on the left of the diagram. The supply is adjusted so that the levels in each container are at the same constant height throughout. One end of each syphon tube dips into each container so that the rate of liquid supply to the feed tubes is determined by the height of the syphon tube outlet. This in turn is determined by the position of the two eccentric circular cams on which the rigid arms supporting the syphon tubes rest.

The cams are held on a single shaft so that they cannot rotate relative to one another. Further, the cams are opposed so that when one syphon tube is at its greatest height the other is at its lowest. In this way a maximum supply rate of sucrose plus KCl solution is

FIGURE 16

Schematic diagram of apparatus used to produce an oscillation in osmotic pressure (oscillatory concentration of sucrose or mannitol) or I.A.A. concentration as a bathing solution for the plant root.


Fixed Pivot
To Supply Bottles
Eccentric Cams
Containers
Syphon Tubes
Plant
M/30 Sucrose + 10⁻⁴M. KCl
10⁻⁴M. KCl
Nylon Probes
Feed Tubes
Polystyrene Cylinder

delivered to one feed tube while a minimum supply of pure KCl solution is delivered to the other. When the cam shaft is rotated by a half revolution, the pure KCl solution supply rate is at a maximum while the sucrose plus KCl solution supply rate is at a minimum. In this way a constant rate of total solution supply is delivered via the feed tubes syphoning into the small polystyrene cylinder containing the bean root:: set up for extracellular potential measurements. By colouring one of the solution supplies with dye, it has been found that adequate mixing of the two solution supplies, fed to the plant, occurs in the polystyrene cylinder. The potential measuring probes placed at various distances along the plant root are held in holes drilled in the cylindrical container. For intracellular potential experiments, the liquid from the syphon tubes was fed to the perspex mount on which the plant rested. (Section II, 2, Figure 10).

The cam shaft is driven so that it advances by one hundredth of a revolution every time an electric impulse is applied to the drive relay. The electric impulses are produced by an electronic pulse generator, the pulse repetition frequency of which may be adjusted continuously from 2 per minute to 60 per minute. In this way the period of rotation of the shaft and consequently the period of the sucrose (or I.A.A.) concentration oscillation may be varied

from about 1.7 minutes to 50 minutes. However, the range of periods was normally restricted to between 2 and 12 minutes.

The phase of the applied oscillation in concentration (sucrose or I.A.A.) is recorded on the potential chart, either for extracellular or intracellular measurements, by automatically applying a voltage sufficient to deflect the pen to an off-scale position when the supply rate of sucrose (or I.A.A.) solution maximises. The total solution supply rate remains approximately constant (20 c.c. per minute) throughout the cycle. The volume of the cylinder surrounding the plant root, for extracellular recording, is only about 1 c.c., and for intracellular measurements, the channel containing the root is again about 1 c.c. Hence there is only a negligible phase difference (a few degrees) between the concentration round the plant and the solution supply even for periods as short as 2 minutes. Further, it may be shown that the oscillation in concentration deviates from the nominal value (0 to M/30 for sucrose or mannitol, and 0 to 10^{-9}, 10^{-7} or 10^{-5}M for I.A.A.) by less than 1 percent even for the 2 minute period. The delay involved in the syphon feed tubes has been found to be negligible (about 1 second).

In some series of experiments it was required to change the constitution of the plant root's bathing solution very rapidly. Once again, since the solution supply rate was 20 cc/min. and the

volume of the plant root container only 1 c.c. (for either intracellular or extracellular measurements), this operation could be accomplished after about one twentieth of a minute. In some of the intracellular experiments it was desirable to reduce this time constant still further. Consequently in such experiments the channel length was reduced and the flow rate of the solution increased so that the change could be accomplished in less than a second. In most experiments however this was unnecessary.

3. Potential Variations Evoked by Sudden Changes in Osmotic Pressure and Auxin Concentration.

When the osmotic pressure of the bathing solution surrounding the plant root is suddenly changed, there are two distinct components in the resulting variation of the plant's intracellular potentials. The first is a rapid change to a new steady level of potential. Following this effect, the time-course of which is only a matter of seconds, there is a much slower potential variation lasting for several minutes. This slow component often exhibits damped oscillations of period similar to the spontaneous type described previously in Section I, 3 and 4. The extracellular potential does not show the rapid component, only a slow variation similar to that for the intracellular potential appears.

Figure 17 shows the rapid intracellular potential response to sudden changes in osmotic pressure. These potentials, recorded with respect to a distant reference, were measured in the first cortical cell past the epidermis at three regions of the root, viz. meristematic (1.5 mm), elongating (5 mm) and mature cells (15 mm). In all three regions a sudden increase in osmotic pressure causes a rapid increase in the potential magnitude, i.e. the potential becomes more negative. The potential then reaches a new steady value, the time constant involved being only about three to five seconds. When the osmotic pressure is decreased the potential returns to its original value again with a time constant of three to five seconds.

This effect is readily explained in terms of the following general considerations. The salt concentration inside plant cells is relatively strong compared with the bathing medium outside (10^{-4}M KCl). This difference in ionic concentration results in the negative potential of the plant cell's interior. If the osmotic pressure of the bathing medium be increased, water is extruded from the cell, so increasing the salt concentration of its interior. This then leads to an increase in the electro-negativity of the cell's interior with respect to the outside.

FIGURE 17

The time-course of the rapid intracellular potential response to sudden changes in osmotic pressure. These potentials, recorded with respect to a distant reference, were measured in the first cortical cell past the epidermis at three regions of the root, viz. meristematic (1.5 mm. from the tip), elongating (5 mm) and mature cells (15 mm).

The upward-pointing arrows indicate a change in molarity of sucrose from 0 to M/30 (increase in osmotic pressure) while those pointing down indicate a decrease from M/30 to 0.

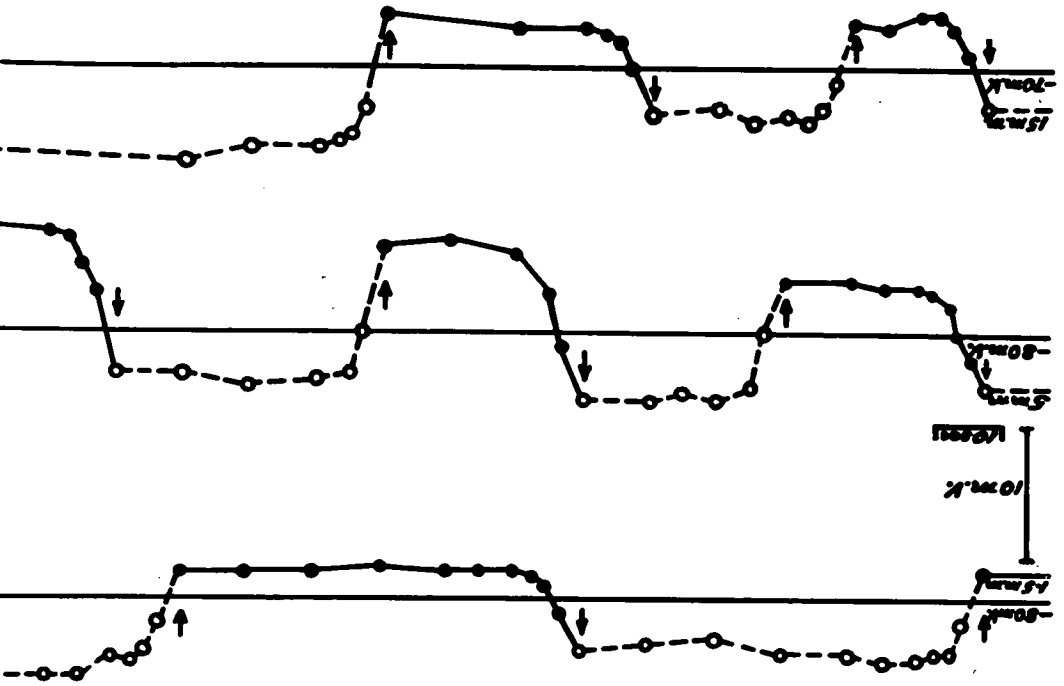


Figure 18 shows the potential changes at various depths in the tissue caused by osmotic pressure changes in the external bathing solution. The potentials were recorded 3 mm from the root tip and were measured with respect to a reference probe placed just outside the root surface in the external bathing solution near where the probe insertion was made. In this way only the potential across the tissue was measured.

In Figure 18 it is seen that the potential changes, caused by osmotic pressure changes, decrease with depth in the tissue. Further, the onset of the potential change appears to be delayed as the depth in the tissue increases. However even at a depth of 100μ the potential change occurs quickly, with very little delay (time constant of 3 to 5 seconds) before attaining a new steady value.

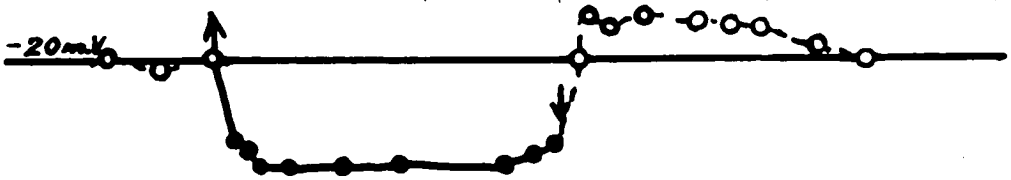
As well as these rapid intracellular potential changes evoked by sudden osmotic pressure changes, there are other slow variations which last for several minutes rather than seconds. This is shown in Figure 19. After the initial rapid change in potential, a further variation occurs in which irregular, damped oscillations frequently appear. This slow variation tends to oppose the initials sudden change and eventually, after several minutes, the potential returns to a value closer to that before the osmotic pressure change occurred. This suggests that there is some process

FIGURE 18

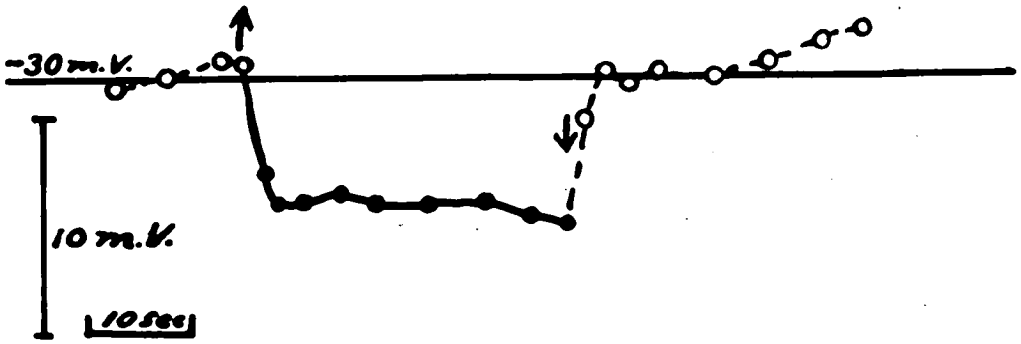
The time-course of the rapid transverse tissue potential response to sudden changes in osmotic pressure. These potentials were measured 3 mm. along the root from the tip at varying depths in the tissue.

The upward-pointing arrows indicate a change in molarity of sucrose from 0 to M/30 (increase in osmotic pressure) while those pointing down indicate a decrease from M/30 to 0.

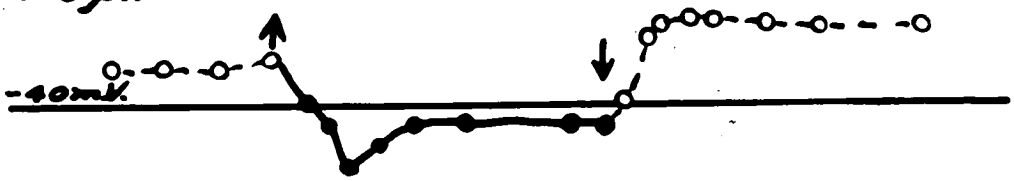
20 μ (epidermis)



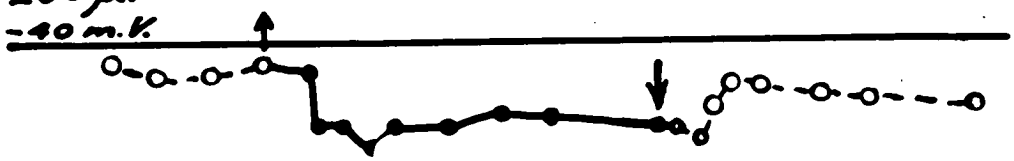
50 μ. (cortex)



100 μ.



200 μ.



300 μ.

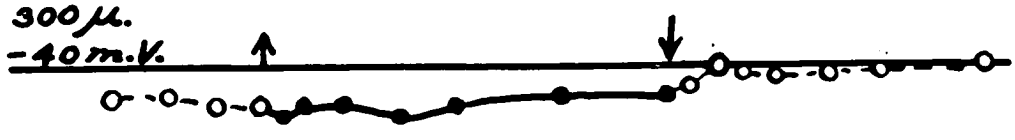
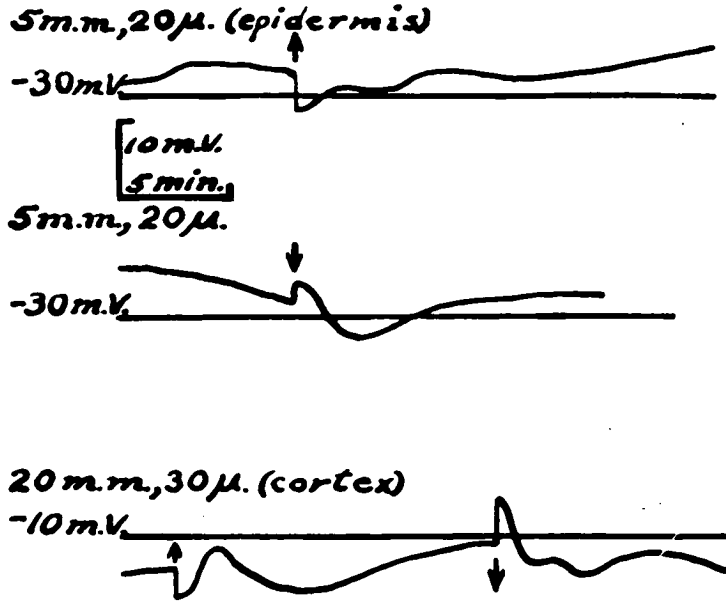


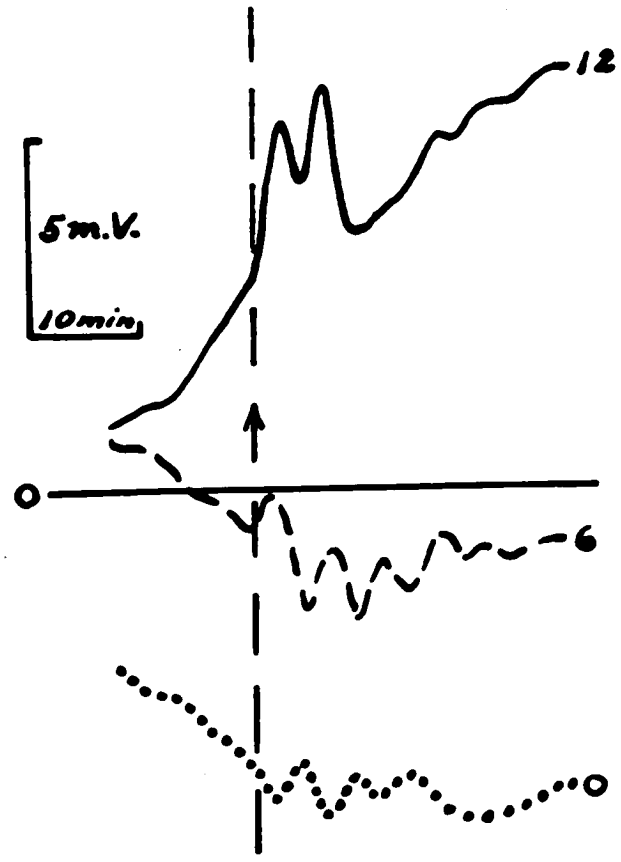
FIGURE 19

The time-course of the slow intracellular potential response to sudden changes in osmotic pressure. These potentials were measured in the epidermis and the cortex at the distances along the root from the tip as shown. The changes in osmotic pressure are indicated by arrows exactly as in Figures 17 and 18.

FIGURE 20

The time-course of the extracellular potential response to a sudden change in osmotic pressure (molarity of sucrose solution changed from 0 to M/30 at dashed line). The potentials were measured at points adjacent to the root at the distances (in m.m.) from the root tip as shown on the potential traces.


5m.m., 20μ. (epidermis)
-30mV.
10m.V.
5min.
5m.m., 20μ.
-30m.V.
20m.m., 30μ. (cortex)
-10m.V.


12
5m.V.
10min.
0
-6
0

which controls the potential at a certain value. These slow potential variations are comparable in magnitude to the rapid initial change. The distribution of their magnitudes throughout the tissue follows the same pattern as that for the rapid changes. That is, the magnitudes are largely independent of position along the length of the root but decrease with depth in the tissue.

Figure 20 shows the extracellular potential response to a sudden change in the osmotic pressure of the bathing solution. There is no rapid change as for the intracellular potentials but the slow variation with damped oscillations still appears. Since the extracellular potentials are caused purely by the flow of bioelectric current through the resistive load of the bathing solution, it seems reasonable to associate the rapid component with the non-ohmic component of the potentials across the plant tissue and the slow component with the ohmic potentials maintained by bioelectric currents both inside and outside the root.

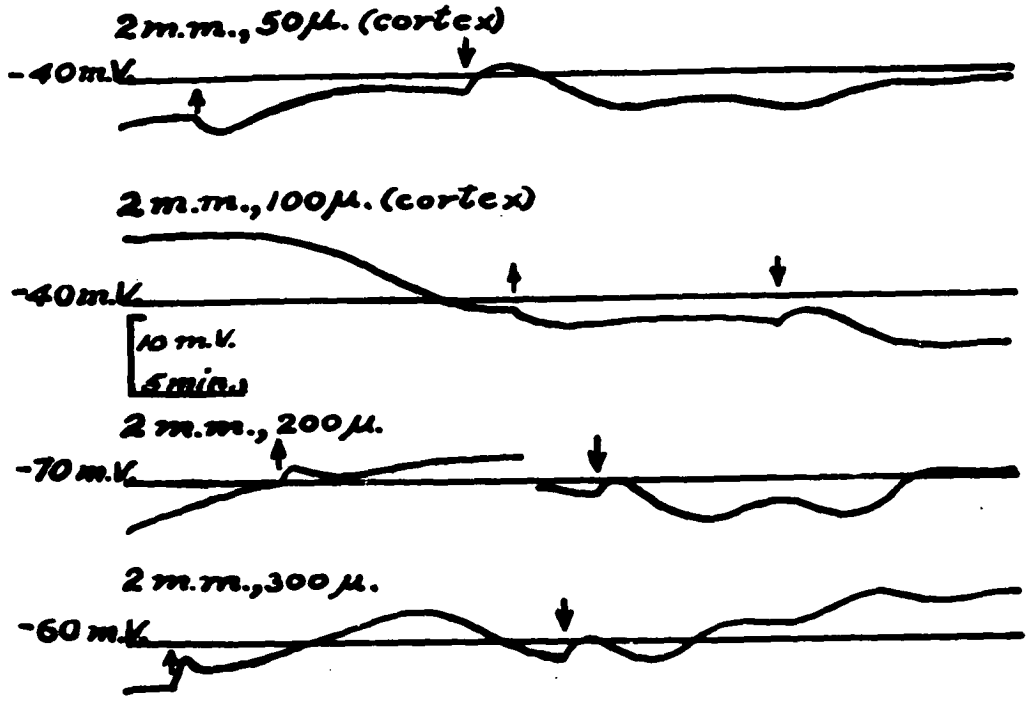
When the concentration of the auxin I.A.A. in the root's bathing solution is suddenly changed, there is no rapid change in either the intracellular or the extracellular potentials. However there are slow variations similar to those for osmotic pressure changes, again often exhibiting damped oscillations. This is shown in Figure 21 for the intracellular potentials. These variations again occur at all positions along the root but the oscillations are often

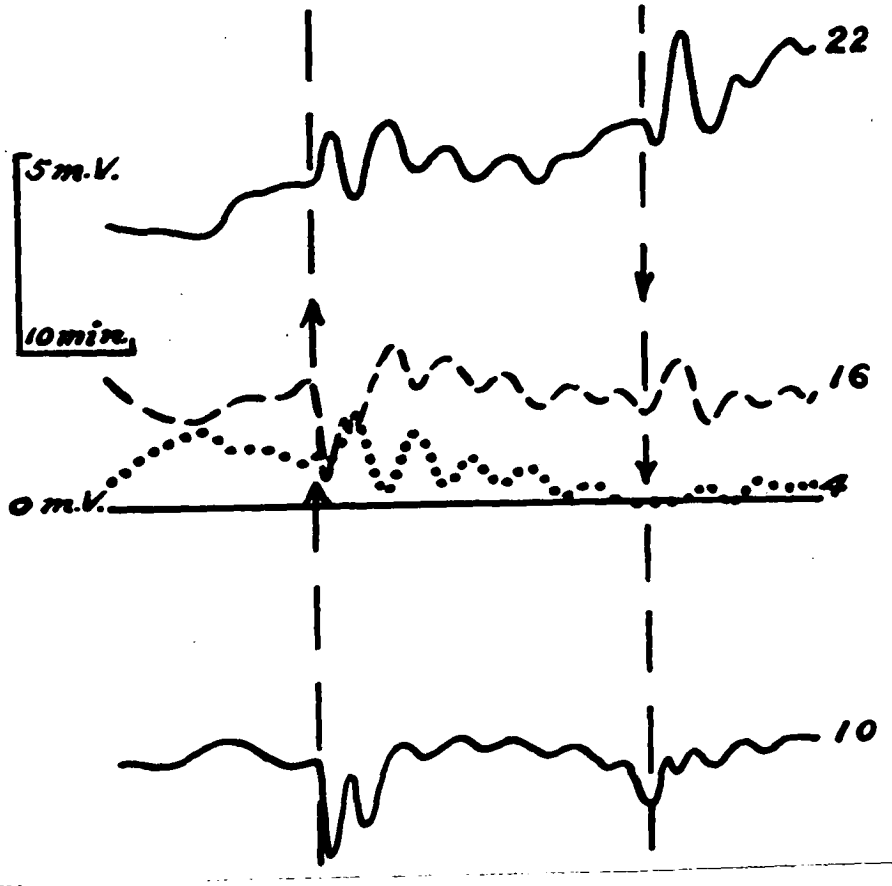
FIGURE 21

The time-course of the intracellular potential response to sudden changes in I.A.A. concentration. The upward-pointing arrows indicate a change from 0 to 10^{-7}M I.A.A. in the root's bathing solution, while those pointing down indicate a change from 10^{-7}M to 0.

FIGURE 22

The time-course of the extracellular potential response to a sudden change in I.A.A. concentration. These potentials were measured at points adjacent to the root at the distances (in m.m.) from the root tip as shown on the potential traces. The changes in I.A.A. concentration are shown by the arrows (at the vertical dashed lines) exactly as in Figure 21.


2 m.m., 50 μ. (cortex)
-40 m.V.
2 m.m., 100 μ. (cortex)
-40 m.V.
10 m.V.
5 min.
2 m.m., 200 μ.
-70 m.V.
2 m.m., 300 μ.
-60 m.V.


22
5 m.V.
10 min.
16
0 m.V.
4
10

more marked at the elongating region between 3 and 5 mm. from the root tip.

Figure 22 shows the extracellular response to sudden changes in the I.A.A. concentration of the bathing solution. These potentials were recorded simultaneously at various positions along the length of the root. The potential variations are similar to those recorded intracellularly, there again being no rapid components. The oscillations appearing in the slow component of the potential variations following a sudden change in osmotic pressure or auxin concentration are similar to those in transient potentials evoked by stimulating the plant in many other ways (Section I,4). When spontaneous potential oscillations occur it is found that they are of the same period as the damped oscillations appearing in transient potentials caused by any of the stimuli described in Section I, 4, including changes in osmotic pressure or auxin concentration.

4. Potential Response to Applied Oscillations in Osmotic Pressure and Auxin Concentration

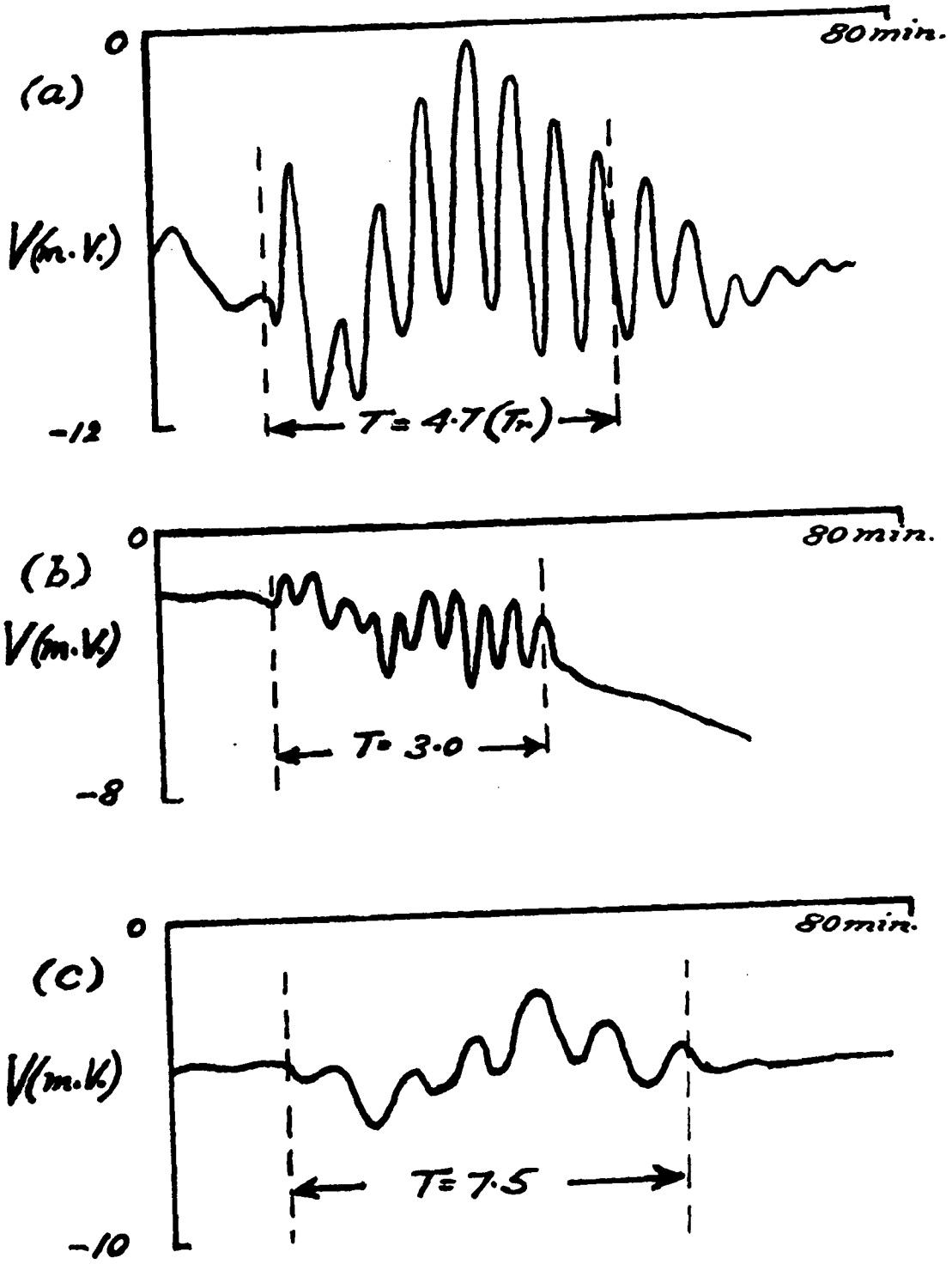
The effects of applying oscillations in the osmotic pressure or the concentration of I.A.A. are qualitatively similar in so far as the bioelectric potentials are concerned. For the range of periods used, i.e. from about 2 minutes to 20 minutes, both the intracellular and the extracellular potentials are forced to oscillate

FIGURE 23

(a) The extracellular potential response to an osmotic pressure oscillation, the period of which is close to the natural period of oscillation for the plant root.

(b) and (c) The extracellular potential responses to osmotic pressure oscillations of periods considerably less than (b) and greater than (c) the natural period of oscillation for the plant root.

In all three cases the potentials were measured at the actively resonant (elongating) region of the same plant root. The osmotic pressure was oscillated between 0 and M/30 for the time interval between the vertical dashed lines in all three cases.


(a)
0
80 min.
V(m.V.)
-12
T = 4.7 (Tn.)
(b)
0
80 min.
V(m.V.)
T = 3.0
-8
(c)
0
80 min.
V(m.V.)
T = 7.5
-10

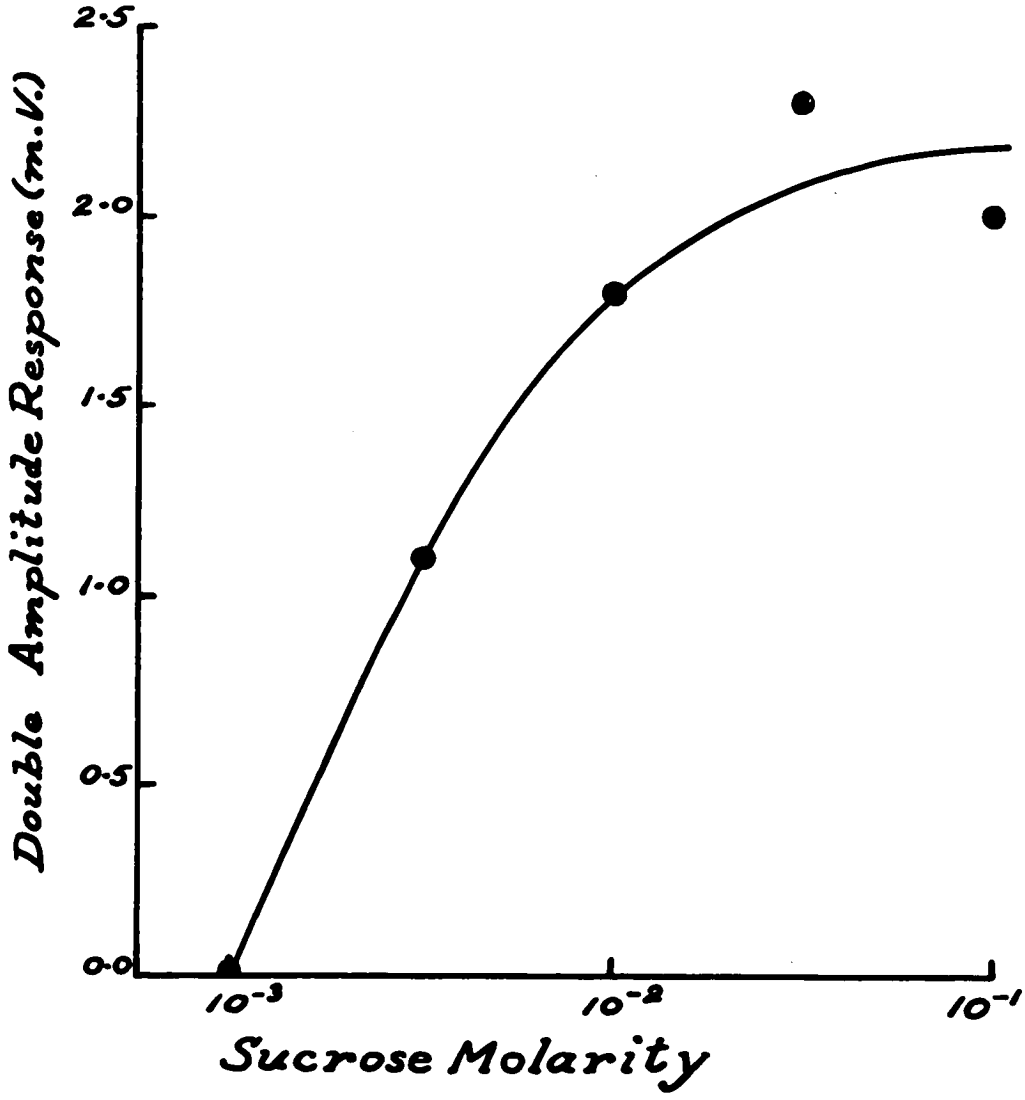
at the same period as the applied oscillation in osmotic pressure or I.A.A. concentration. Figure 23(a) shows the extracellular potential response to the application of an osmotic pressure oscillation the period of which is close to the natural period of oscillation for the plant. After removing the osmotic pressure oscillation the potential oscillation is seen to continue for a number of cycles though it is damped. Moreover the amplitude response is much greater than that in Figures23(b) and (c) where the applied period of the osmotic pressure oscillation is considerably different from that of the plant's natural potential oscillations. In such cases (Figures23 (b) and (c)), the potential oscillation disappears as soon as the applied oscillation is removed. The resonance effect at the natural period (Figure 23(a)) is most marked in the region of cell elongation in the root.

For intracellular potentials the corresponding results are very similar to those described above for extracellular potentials, the same resonance effect occurring in the elongating region of the plant root.

The amplitude of the potential response depends on the amplitude of the applied osmotic pressure oscillation at constant period. Figure 24 shows the average double amplitude response to oscillations in the molarity of the sucrose solution bathing the plant root.

FIGURE 24

The relation between the double amplitude of the oscillatory potential response and the applied amplitude of the osmotic pressure oscillation. The sucrose concentration was oscillated betwenn 0 and the peak molarities. The oscillatory period (3.8 min) was less than the natural period for the plant. The extracellular potential was measured at the actively resonant or elongating region of the root.


Double Amplitude Response (m.V.)
2·5
2·0
1·5
1·0
0·5
0·0
10⁻³
10⁻²
10⁻¹
Sucrose Molarity

The KCl concentration was maintained at 10^{-4}M throughout and the sucrose concentration was oscillated between zero and the peak values shown. The period of the osmotic pressure oscillation was 3.8 minutes throughout. For oscillations in sucrose concentration with peak values lower than 10^{-3}M the extracellular potential response is negligible. Between 10^{-3}M and 3×10^{-2}M the potential response increases with molarity and above 3×10^{-2}M it remains constant. Since the potential response does not increase beyond M/30 and since this molarity is well below the plasmolysis threshold it has been chosen as the maximum osmotic pressure in most experiments.

The intracellular potential responses to applied oscillations in osmotic pressure have been determined as a function of position in the root tissue. Figures 25 (a) and (b) show the potential response at varying depths in the tissue situated at 5 mm. from the root tip. In Figure 25(a) the intracellular potentials were recorded with respect to a probe close to the surface of the root but still in the bathing solution near where the measuring probe was inserted, i.e. these potentials are the transverse tissue potentials. The top trace in Figure 25(a) however was recorded extracellularly with respect to a distant reference electrode. In Figure 25(b) the potentials, both extracellular (top pair) and intracellular were measured with respect to a distant reference. The data in

FIGURE 25

The time-courses of the transverse tissue potentials (a) and the intracellular potential with respect to a distant reference (b) in response to oscillations in osmotic pressure (0 to M/30) recorded at varying depths in the tissue, at 5 mm. along the root from the tip. The top trace in (a) and the first two traces in (b) were however recorded extracellularly with respect to a distant reference.

The data in (a) was obtained from a different plant from that of (b), and two slightly different applied periods of osmotic pressure oscillation were used for the two plants. However in each case the applied period was considerably less than the natural period of potential oscillation for the plant.

The time at which the maximum osmotic pressure (M/30) occurred in the cycle is indicated by the short vertical strokes on the potential traces.

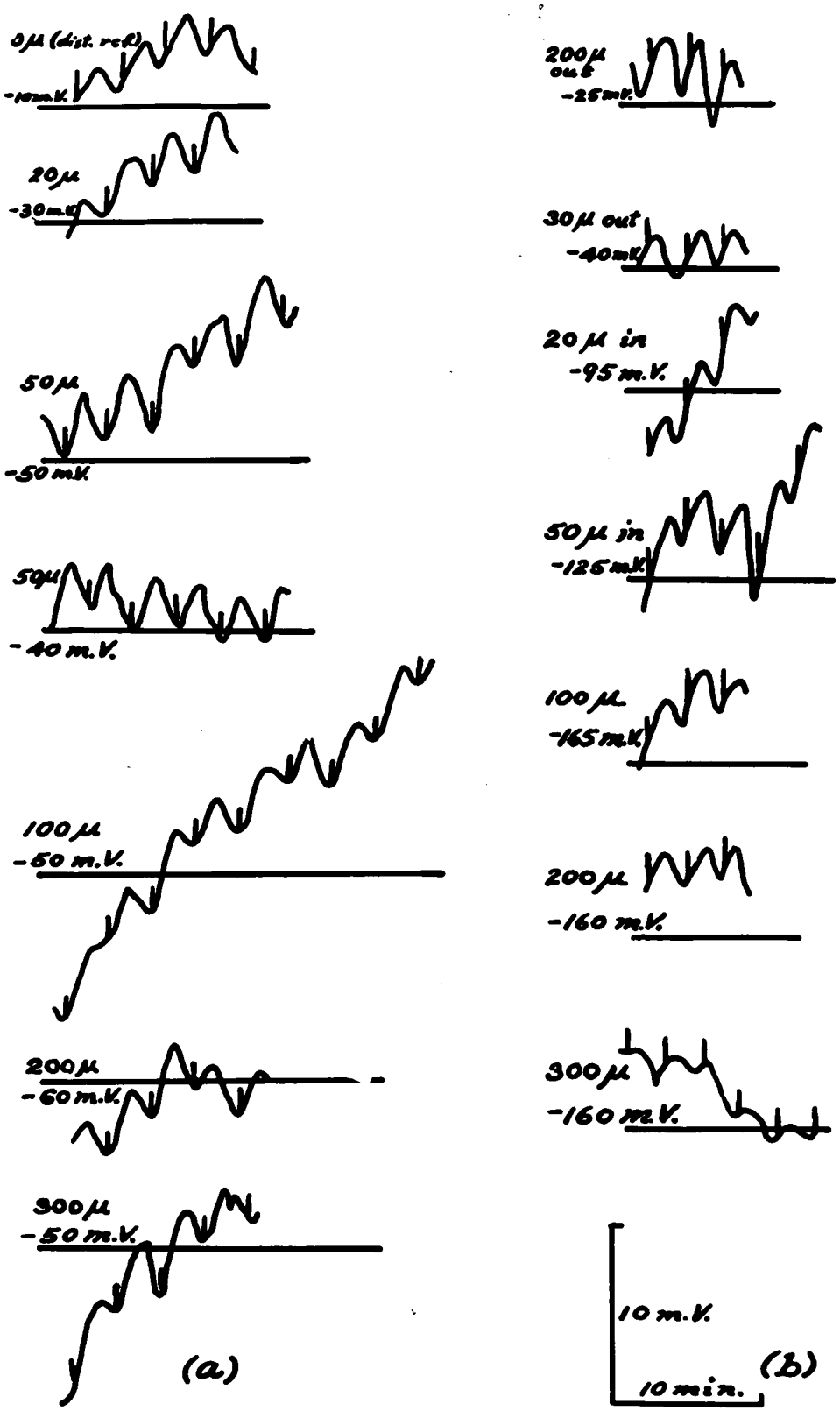

0μ (dist. ref.)
-10m.V.
20μ
-30m.V.
50μ
-50 m.V.
50μ
-40 m.V.
100μ
-50 m.V.
200μ
-60 m.V.
300μ
-50 m.V.
(a)
200μ out
-25m.V.
30μ out
-40m.V.
20μ in
-95 m.V.
50μ in
-125m.V.
100μ
-165m.V.
200μ
-160 m.V.
300μ
-160 m.V.
10 m.V.
10 min.
(b)

Figure 25(a) was obtained from a different plant from that of Figure 25(b), and two slightly different applied periods of osmotic pressure oscillation were used for the two plants. However in each case the applied period was considerably less than the natural period of potential oscillation for the plant. The time at which the maximum osmotic pressure occurred in the cycle is indicated by the short vertical strokes on the potential traces.

The phase of the extracellular potential oscillations in Figures 25(a) and (b) are substantially the same, the potential lags the osmotic pressure by about 90°. However the phase of the transverse tissue potentials (Figure 25(a)) is quite different from that of the extracellular potentials, the transverse tissue potential oscillations being in antiphase with the osmotic pressure oscillations. That is, the magnitude of the transverse tissue potential maximises at the same time as the osmotic pressure. This is in agreement with the results obtained for sudden changes in osmotic pressure. Further, this phase relation is largely independent of depth in the tissue.

The phase response of the intracellular potentials, recorded with respect to a distant reference (Figure 25(b)) is different from that of the transverse tissue potentials and is more like the phase of the extracellular potentials. This is to be expected since

the intracellular potential with respect to a distant reference is the sum of the appropriate transverse tissue potential and the extracellular potential (Section II,2).

The amplitude response in Figures25(a) and (b) reaches a maximum at a depth of 50μ, i.e. in the outer cortical cells. Figures26(a) and (b) again show the phase and amplitude responses to osmotic pressure oscillations of a constant period considerably less than the natural period of the plant. These potentials were recorded at various positions along the plant root in both cases. Figure 26(a) shows the transverse tissue potentials, all recorded at a depth of 50μ, while in Figure 26(b) the intracellular potentials, with respect to a distant reference, were recorded at a depth of 100μ.

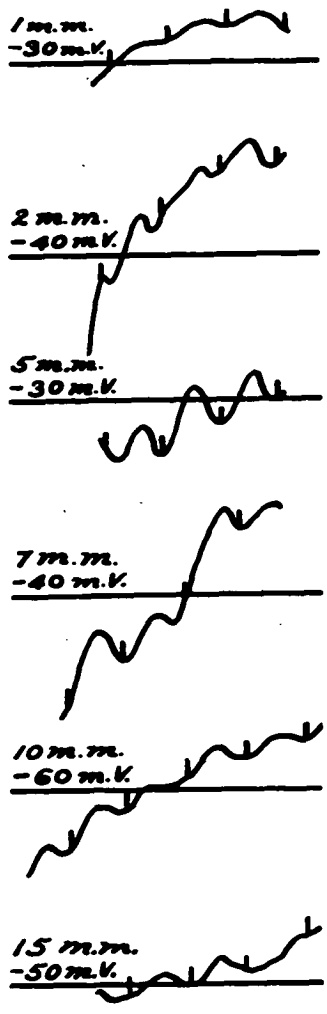
The phase responses are again largely independent of position in the tissue, the transverse tissue potentials being in antiphase with the osmotic pressure while those in Figure 26(b) show a different phase response which is however similar to that of Figure 25(b).

The amplitude response maximises at about 5 mm from the tip in both cases. In Figures 27(a) and (b) the amplitude response data of Figures 25 and 26 are shown as functions of position in the tissue. In Figure 27(a) both intracellular potential responses maximise at 50μ while in Figure 27(b), both maximise at 5 mm from

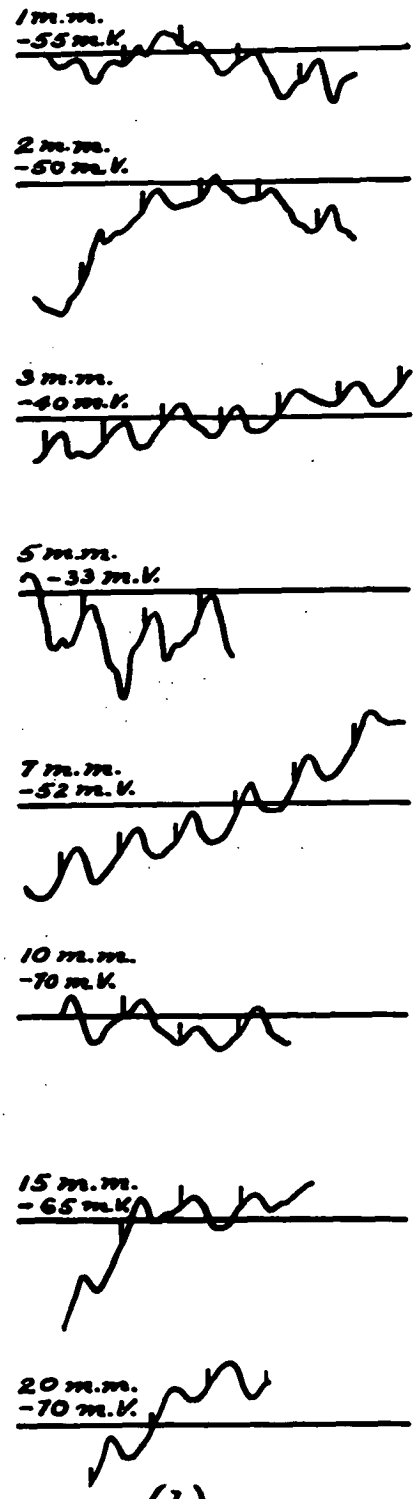
FIGURE 26

The responses of the transverse tissue potentials (a) and the intracellular potential with respect to a distant reference (b) evoked by oscillations in osmotic pressure (0 to 1/30) recorded at varying distances along the root from the tip at depths of 50μ in (a) and 100μ in (b). The same period of osmotic pressure oscillation (less than the natural period of oscillation for the plant) was used throughout (a) and (b).

The vertical strokes on the potential traces have the same meaning as in Figure 25.


1 m.m.
-30 m.V.
2 m.m.
-40 m.V.
5 m.m.
-30 m.V.
7 m.m.
-40 m.V.
10 m.m.
-60 m.V.
15 m.m.
-50 m.V.
10 m.V.
10 min.

(a)

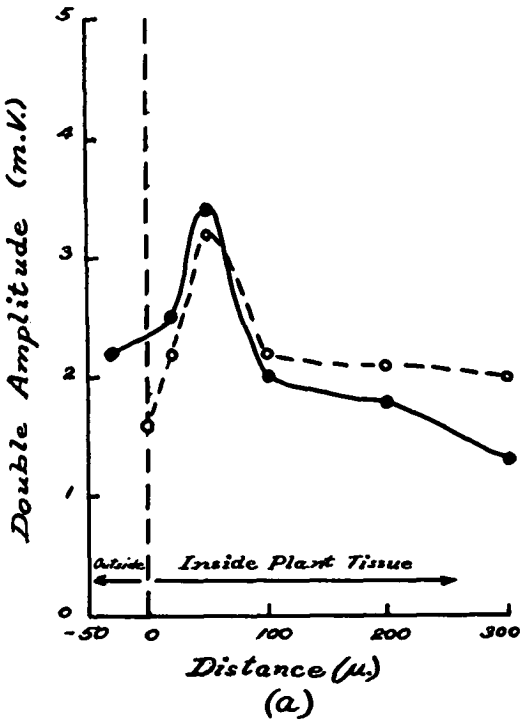

1 m.m.
-55 m.V.
2 m.m.
-50 m.V.
3 m.m.
-40 m.V.
5 m.m.
-33 m.V.
7 m.m.
-52 m.V.
10 m.m.
-70 m.V.
15 m.m.
-65 m.V.
20 m.m.
-70 m.V.

(b)

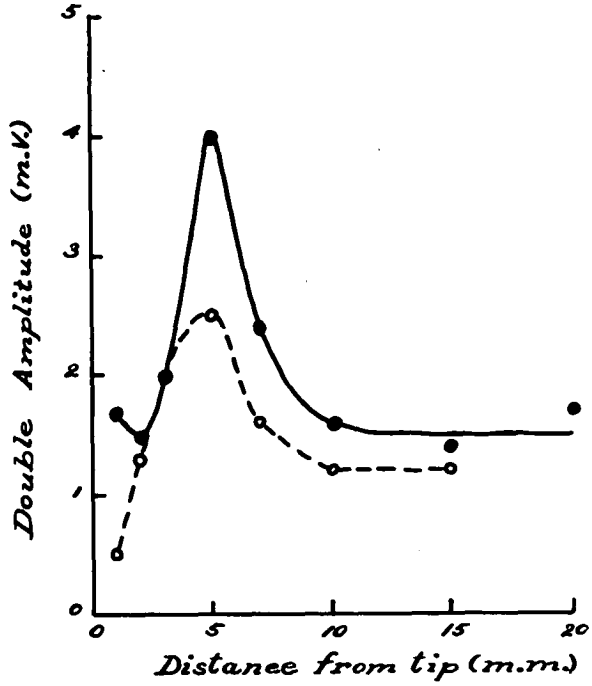
FIGURE 27

(a) The relation between the double amplitude oscillatory response of the transverse tissue potential (dashed line, open circles), the intracellular potential with respect to a distant reference (full line, black circles) and the distance inside the root tissue (depth) recorded at 5 mm. from the root tip. (Data obtained from Figure 25).

(b) The relation between the double amplitude oscillatory response of the transverse tissue potential (dashed line, open circles), the intracellular potential with respect to a distant reference (full line, black circles) and the distance along the root from the tip, recorded at depths of 50μ (dashed line, open circles) and 100μ (full line, black circles). (Data obtained from Figure 26).


Double Amplitude (m.V.)
5
4
3
2
1
0
-50
0
100
200
300
Outside
Inside Plant Tissue
Distance (μ.)

(a)


Double Amplitude (m.V.)
5
4
3
2
1
0
0
5
10
15
20
Distanee from tip (m.m.)

(b)

the tip. These results are similar to those of Section II, 6, in which it was found that spontaneous oscillations of maximum amplitude appeared in the outer cortex of the root's elongating region.

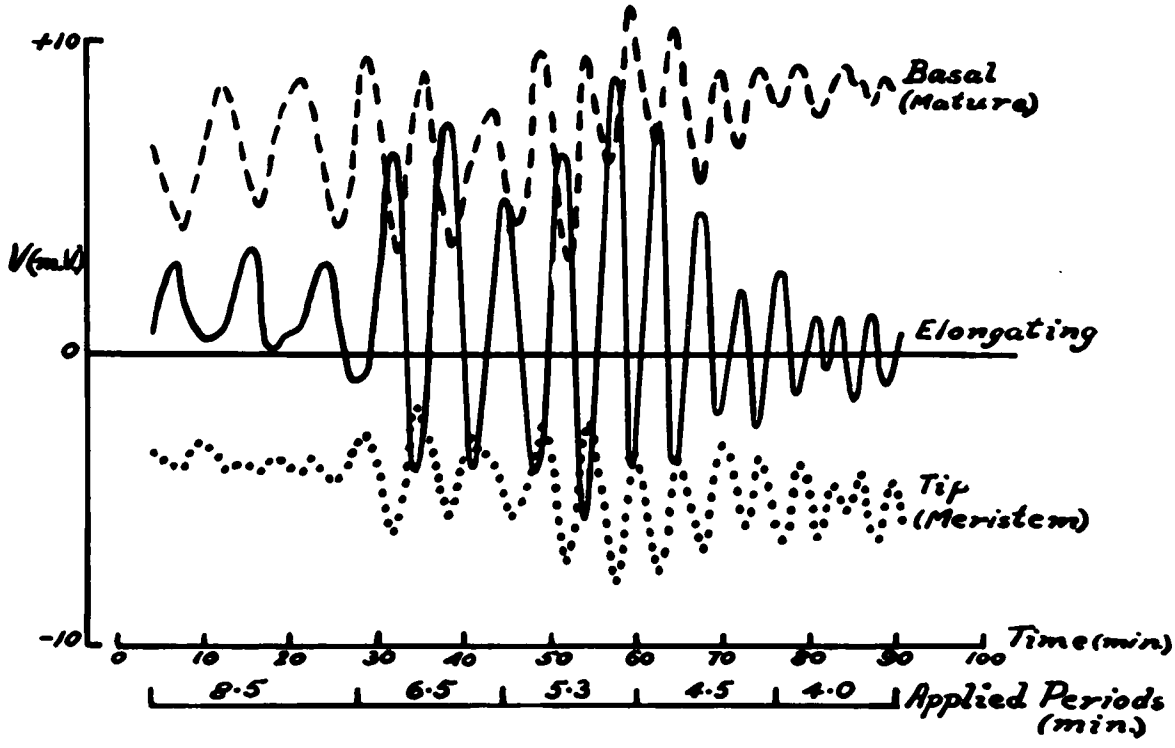
5. The Resonant Response to Oscillations in Osmotic Pressure and Auxin Concentration and its Relation to the Plant's Morphology

In Figure 28 the extracellular potential responses to five different applied periods of osmotic pressure oscillation are shown. These potentials were recorded simultaneously at three different regions along the root. At all three positions, a potential oscillation is produced, its period being equal to that of the applied osmotic pressure oscillation. The oscillatory response is greater for some periods than for others, this being particularly true for the potential recorded at the elongating region.

It is generally found that the amplitude response of the potential at the elongating region of the root shows a marked resonance, the period at which the resonance occurs being the natural period of potential oscillation which is observed in the plant's transient potentials. (Scott, 1957; Jenkinson, 1958 and Section I,4). At other regions along the root such as the tip, primary meristem and basal regions, the resonance is not so marked. Further, the oscillatory potentials at these regions are substantially

FIGURE 28

The extracellular potential responses to oscillations in the osmotic pressure of the root's bathing solution (0 to M/30 sucrose solution) at different periodicities (shown below the figure) at three regions of the root viz. tip (meristematic), elongating and basal (mature).


+10
V(mV)
0
-10
Basal
(Mature)
Elongating
Tip
(Meristem)
0
10
20
30
40
50
60
70
80
90
100
Time (min)
8·5
6·5
5·3
4·5
4·0
Applied Periods
(min)

in antiphase with those produced at the actively resonant elongating region. It appears that these other regions are not so actively resonant and that their potential oscillations are of a passive type caused largely by return currents produced in the actively resonant elongating region. It will be recalled that the plant tissue exhibiting spontaneous intracellular oscillations of maximum amplitude was also the elongating region of the root (Section II,6).

By means of the following experiments it has been possible to test the hypothesis that the actively resonant region is located in the elongating zone of the root (between 2 and 12 mm. in the bean roots used) and not in the primary meristem nor in the regions where cell elongation has ceased. First the osmotic pressure oscillation was set at the resonant (or natural) period. This caused the plant to produce enhanced oscillations at the same period. Three millimetres of tissue from the tip end of the root were then cut away thus removing the primary meristem. This treatment did not affect the response of the plant to the resonant period in any way, within one or two hours of the excision. However the removal of a further 10 mm. greatly inhibited the response to the resonant period, and in some cases completely supressed the plant's potential response to the osmotic pressure oscillation.

In some experiments the resonant condition was first evoked with most of the root (about 3 cm.) immersed in the bathing solution. Then the plant was raised so that only the last 10 mm. remained in the bathing solution. The resonant oscillations continued although the background potential pattern (i.e. the steady potential pattern on which the oscillations are superimposed) was diminished as in Figure 7 (Section I,3). Excision of the first 3 mm. from the tip end again did not diminish the oscillatory potential response.

This type of experiment was repeated using plants with roots only about 15 mm. in length. In such roots there is hardly any tissue which has ceased elongating. These plants again exhibited strong resonances which were not inhibited by excising the primary meristem. These experiments show convincingly that the elongating zone of the root is the actively resonant region.

Resonant responses, very similar to those of the extracellular potentials to applied oscillations in osmotic pressure, have been observed when the concentration of I.A.A. in the bathing solution is oscillated between zero and 10^{-7}M. If 10^{-9}M I.A.A. is used as the peak concentration in the cycle, resonance still occurs but the response to all periods of oscillation is usually diminished considerably. The response to oscillations in I.A.A. with 10^{-5}M peak concentration is small and no resonance is observed. This is probably because at such high concentrations the elongation of the

root is completely inhibited (Scott, McAulay and Pauline Joyes, 1957). Since oscillations between zero and 10^{-7}M I.A.A. evoke the most marked effect, most of the results for I.A.A. in this section refer to this peak concentration.

For the bioelectric oscillations evoked by I.A.A. oscillations, the actively resonant region is again the zone of elongating cells in the root. The experiments described previously, in which parts of the root were excised while the resonant condition was evoked by osmotic pressure oscillations, were repeated for resonant oscillations evoked by I.A.A. oscillations. Very similar results were obtained showing that only the elongating region is sensitive to auxin oscillations in substantially the same manner as it is to osmotic pressure oscillations.

Routine observations of growth rate using the growth meter described in Section I have shown that in certain isolated instances there are irregular oscillations in the rate of growth of the same period as the resonant potential oscillations evoked by osmotic pressure or I.A.A. However quite frequently marked potential oscillations at resonance have not been accompanied by oscillations in the growth rate.

Almost all the foregoing remarks regarding resonance at the plant's natural period and the position of the actively resonant

region apply equally well to intracellular potential oscillations evoked by applied oscillations in osmotic pressure and I.A.A. concentration. This is true for both the transverse tissue potentials and the intracellular potentials recorded with respect to a distant reference. However it was shown previously (Figures 25 and 26) that for a particular applied period of osmotic pressure oscillation the phase of the transverse tissue potential was different from that of the intracellular potential with respect to a distant reference. It is found also that the phase response of the extracellular potentials differs from the phase response of both types of intracellular potential to osmotic pressure oscillations. Further, both the intracellular and extracellular phase responses differ depending on whether the applied oscillation is in osmotic pressure or I.A.A. concentration. It is now proposed to describe in detail these various phase responses as functions of the applied period.

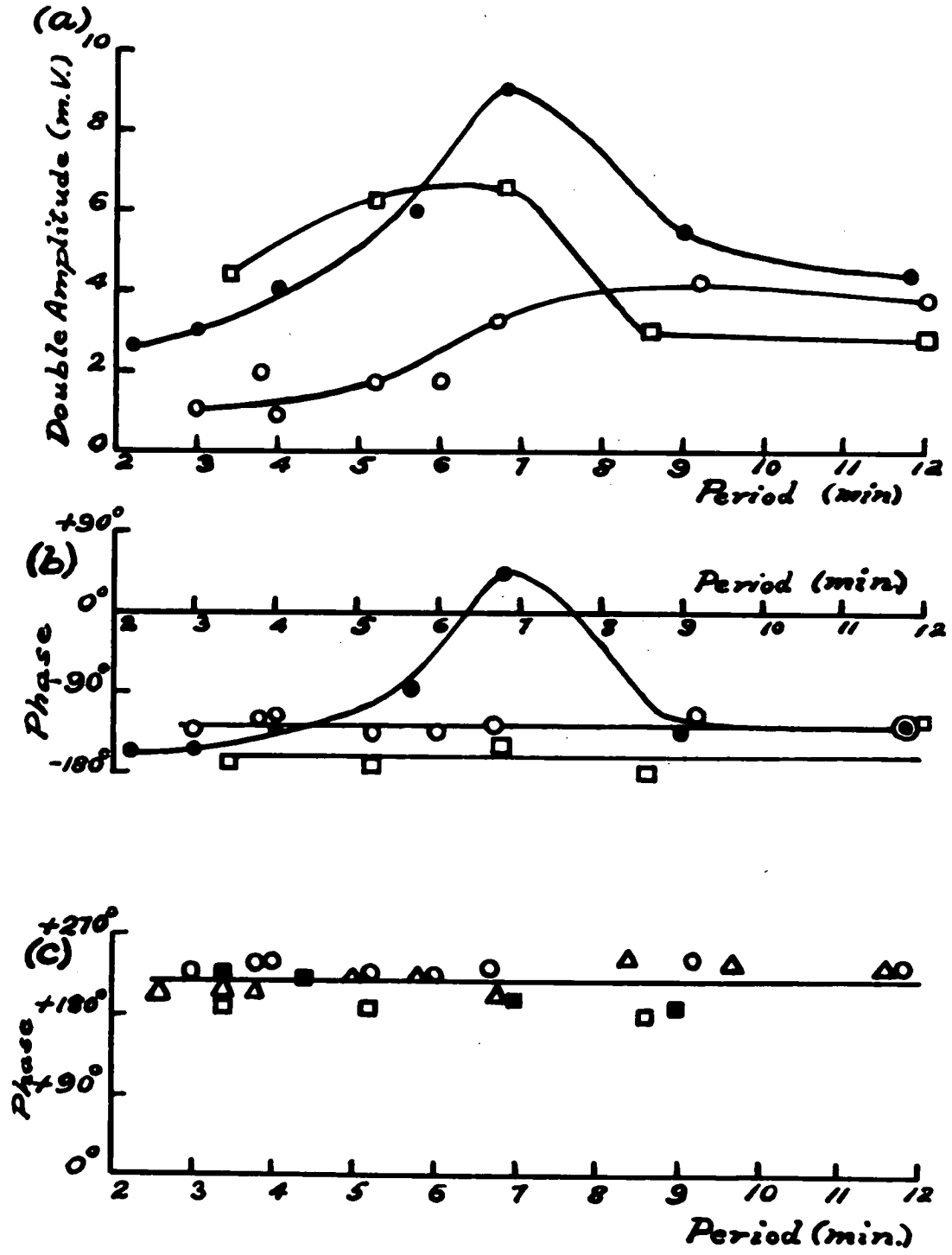
6. The Phase and Amplitude Response to Oscillations in Osmotic Pressure and Auxin Concentration as a Function of Applied Period

Figures 29(a) and (b) show the amplitude and phase response of the intracellular potential, with respect to a distant reference, to osmotic pressure oscillations of varying period. These are typical results obtained from three different plants. The potentials

FIGURE 29

(a) and (b) The relations between the double amplitude (a) and the phase (b) responses of the intracellular potential with respect to a distant reference and the applied period of osmotic pressure oscillation (0 to M/30 sucrose solution) for three plants. The potentials were recorded at 5 mm. from the root tip in all three cases. For two plants (black circles and open squares) the potentials were recorded at a depth of 50μ and for the other (open circles) at 100μ.

(c) The phase responses of the intracellular potentials with respect to a distant reference for a number of plants (different symbols for each) to applied oscillations in osmotic pressure (0 to M/30 sucrose solution).


(a)
Double Amplitude (m.V.)
0
2
4
6
8
10
2
3
4
5
6
7
8
9
10
11
12
Period (min)
(b)
+90°
0°
-90°
-180°
Phase
Period (min)
2
3
4
5
6
7
8
9
10
11
12
(c)
+270°
+180°
+90°
0°
Phase
2
3
4
5
6
7
8
9
10
11
12
Period (min.)

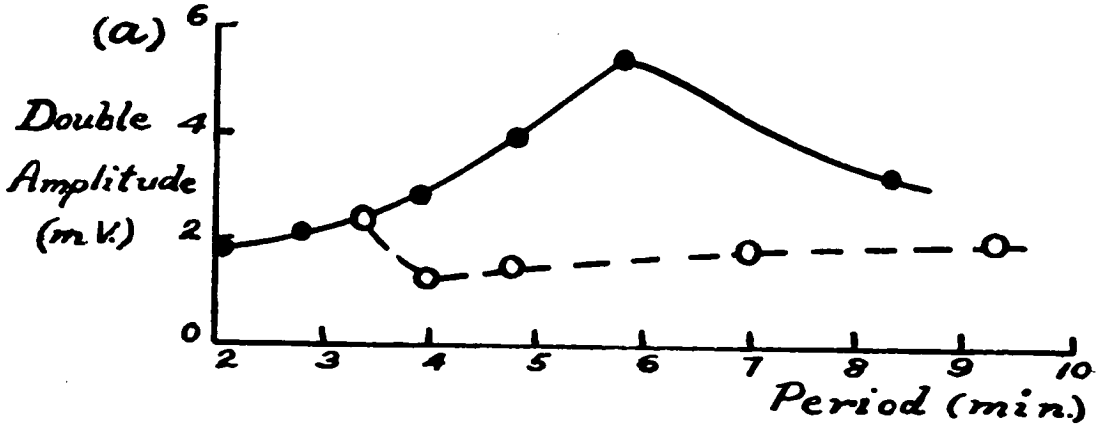
were recorded at depths of 50μ and 100μ all at 5 mm. from the root tip. Two of the amplitude responses exhibit resonances, one stronger than the other, while the other does not. For the less marked of the two resonant cases and for the non-resonant case there is no appreciable change in the phase angle as the period of the applied osmotic pressure oscillation is changed from 3 to 12 minutes. For the more strongly resonant case the phase angle changes from a lag to a slight lead at 6.8 minutes and then at longer periods it returns to a lag, not substantially different from that at short periods.

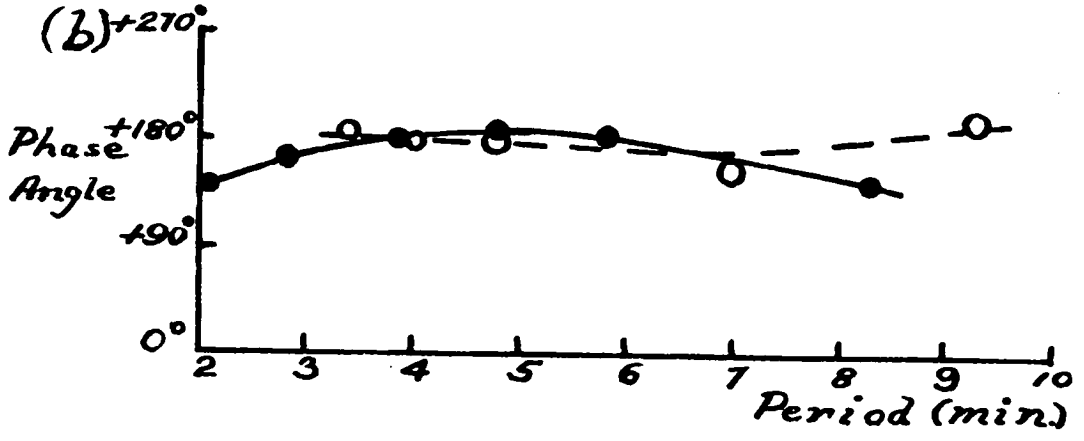
It is generally found that the more strongly resonant cases yield more complex phase responses, such as that in Figure 29(b), than the less resonant cases do. Figure 29(c) shows the phase response data for a number of plants showing little or no resonance. In this case the phase angle is plotted as a lead instead of a lag as in Figure 29(b). The reason for this will be apparent when the phase relations for the transverse tissue potentials and extracellular potentials are described. In Figure 29(c) it is apparent that the phase angle does not change appreciably as the period is varied from about 3 minutes to 12 minutes, passing through the natural periods of oscillation for the plants (usually between 5 and 7 minutes).

Figures 30(a) and (b) show the corresponding amplitude and phase responses for the transverse tissue potentials to oscillations in

FIGURE 30

The relations between the double amplitude (a) and the phase (b) responses of the transverse tissue potentials and the applied period of osmotic pressure oscillation (0 to M/30 sucrose solution) for two plants. In both cases the potentials were recorded at depths of 50μ, 5mm. from the root tip.


(a)
6
Double
4
Amplitude
(m.V.)
2
0
2
3
4
5
6
7
8
9
10
Period (min.)


(b)
+270°
Phase
+180°
Angle
+90°
0°
2
3
4
5
6
7
8
9
10
Period (min.)

osmotic pressure of varying applied period. Results from two typical plants are shown, one of the resonant type and the other showing no apparent resonance. In both plants the potentials were measured at 5 mm. from the root tip and at a depth of 50μ. The potentials were measured with respect to a probe placed close to the root near where the microelectrode insertion was made.

These phase relations are typical of transverse tissue potentials evoked by osmotic pressure oscillations, the potential being substantially in antiphase with the osmotic pressure irrespective of the applied period of oscillation. Even for strong resonances the phase angle does not change with the applied period as it did for the intracellular potentials measured with respect to a distant reference.

This is to be expected since it was found that the delay between a sudden change in osmotic pressure and that in the intracellular potential is only a few seconds (Figures 17 and 18), the potential becoming more negative as the osmotic pressure is increased. Since this time delay is negligible compared with the applied periods of oscillation, the potential would be expected to reach a maximum negative value when the osmotic pressure maximises ~~i.e.~~ the transverse tissue potential oscillation would be in antiphase with the osmotic pressure oscillation.

Although the amplitude response of the extracellular potentials to osmotic pressure oscillations of varying period is essentially the same as that for the intracellular potentials the phase response for the extracellular potentials is fundamentally different. This is illustrated in Figure 31 which shows typical amplitude and phase responses at the actively resonant (elongating) regions for plants subjected to oscillations in osmotic pressure. The natural periods of oscillation, obtained from transient potentials for each of the plants involved, are indicated on the period (T) axis. The resonant period is in agreement with the natural oscillatory period for each plant.

It is apparent that the phase angle changes as the period increases, the greatest rate of phase change being near the resonant period. At short periods, i.e. less than the resonant or natural period, the extracellular potential lags the osmotic pressure by about 90° while at long periods the potential is in antiphase with the applied oscillation in osmotic pressure. The continuous phase change from short to long periods is 270° or three right angles.

In Figure 32 the amplitude and phase responses to applied oscillations in osmotic pressure are shown for typical plants on an harmonic vector response diagram. Again it is evident that there is a change of phase angle of three right angles (approximately) from short to long periods, the rate of change of phase angle with

FIGURE 31

The relations between the double amplitude (a) and the phase (b) responses of the extracellular potentials at the elongating regions and the period of osmotic pressure oscillation (0 to M/30 sucrose solution) for two plants. The natural periods of oscillation for the two plants are shown on the period axis of (a).

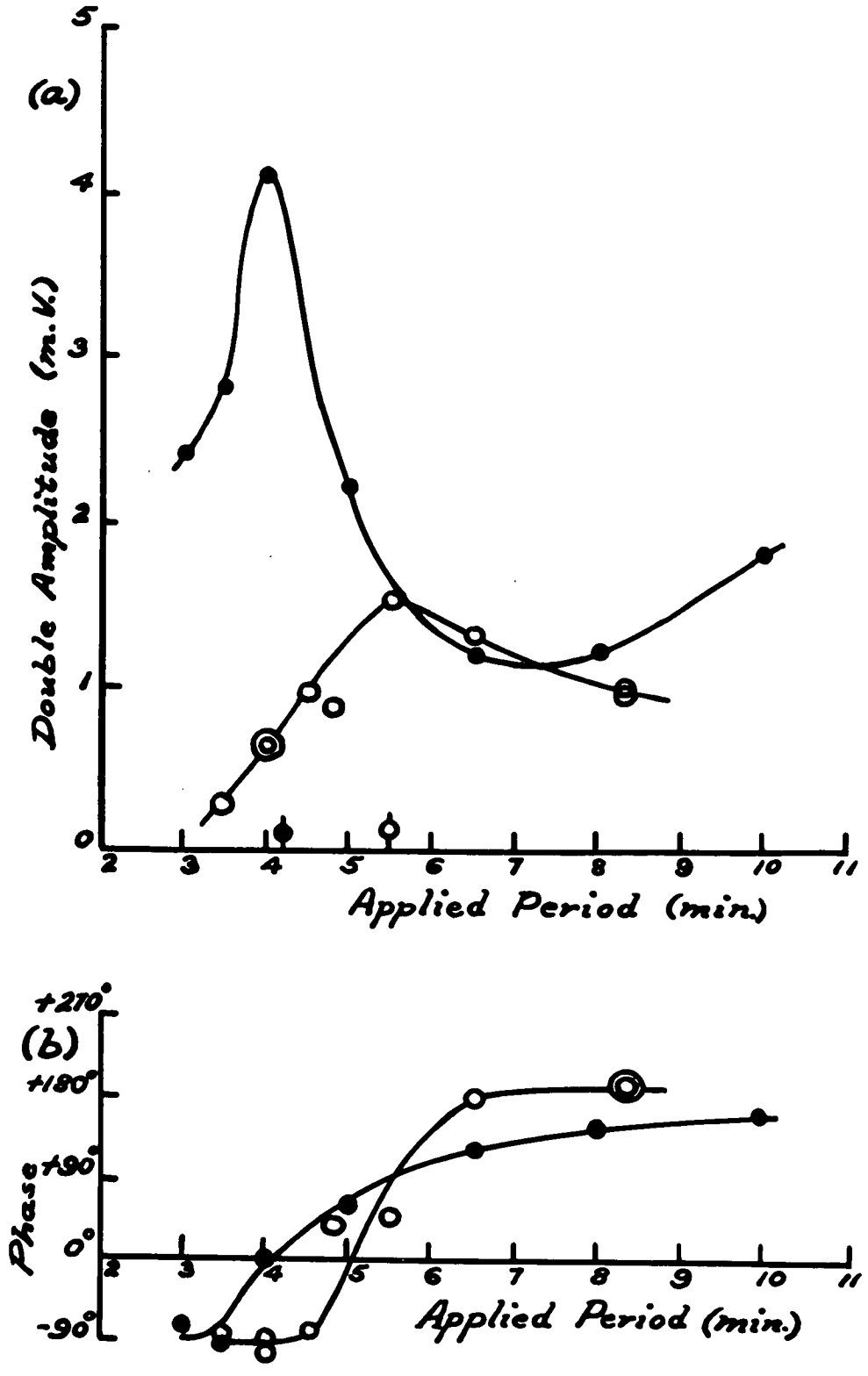
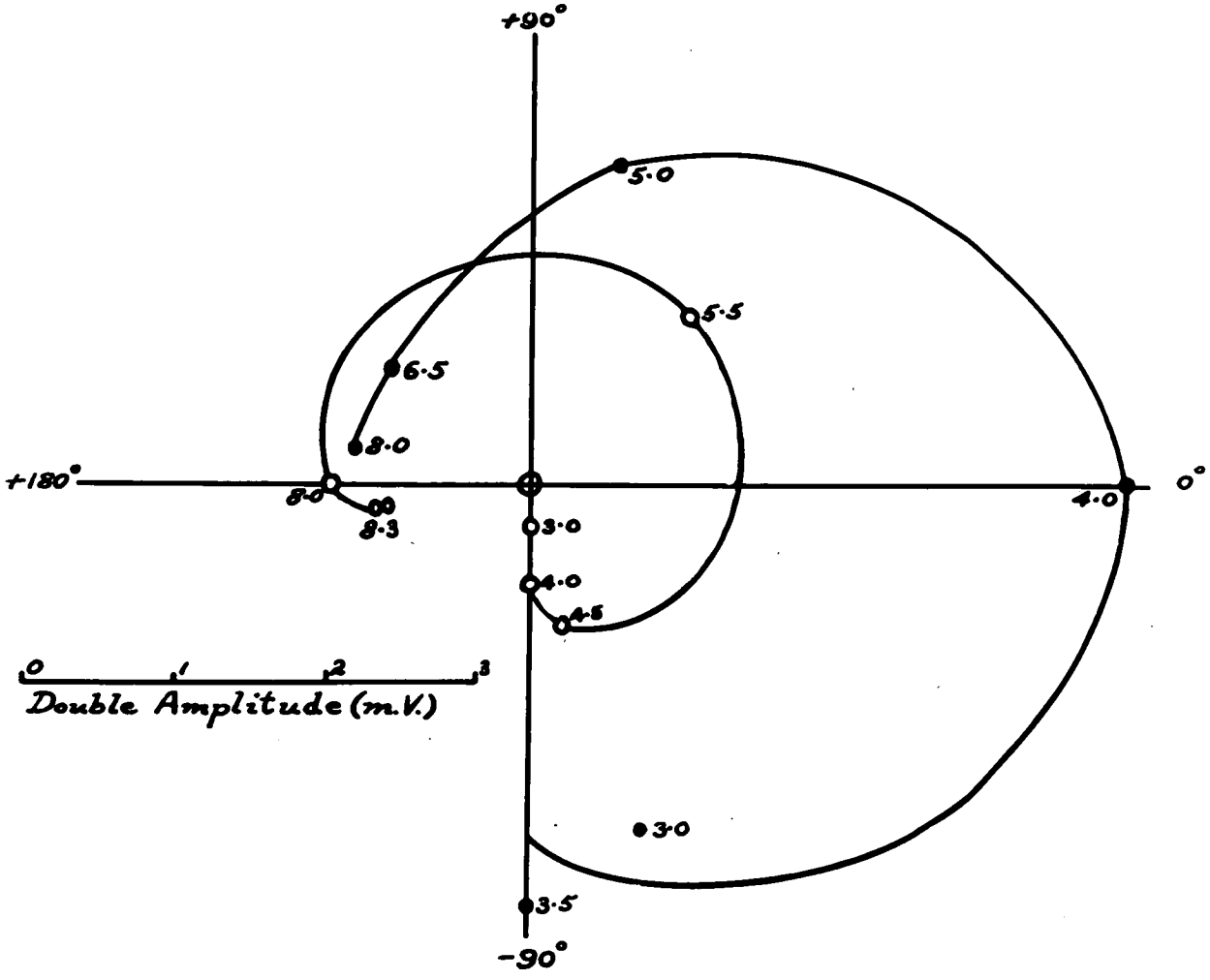

(a)
Double Amplitude (m.k.)
0
1
2
3
4
5
2
3
4
5
6
7
8
9
10
11
Applied Period (min.)
(b)
Phase
+270°
+180°
+90°
0°
-90°
2
3
4
5
6
7
8
9
10
11
Applied Period (min.)

FIGURE 32

Harmonic vector response diagram of the double amplitude and phase responses of the extracellular potentials at the elongating regions of two plants to oscillations in osmotic pressure (0 to M/30 sucrose solution) at the periods (in minutes) shown beside the points plotted.


+90°
5·0
5·5
6·5
8·0
+180°
8·0
8·3
0°
4·0
3·0
4·0
4·5
0
1
2
3
Double Amplitude (m.V.)
3·0
3·5
-90°

period being greatest near the resonance.

Figure 33 shows typical amplitude and phase responses of the extracellular potentials, recorded at the elongating regions, for typical plants subjected to oscillations in I.A.A. concentration. Once again resonances occur at the natural periods, and the phase angle changes rapidly with period near the resonance. However, in contrast to the osmotically evoked potential oscillations, the potential is in phase with the I.A.A. oscillation at short periods although at long periods it is again in antiphase. The change in phase angle from short to long period is thus only two right angles in contrast to three right angles for the extracellular potential response to osmotic pressure oscillations.

In contrast to the corresponding osmotic pressure effects, the intracellular potential response to applied oscillations in I.A.A. concentration is the same as that for the extracellular potential response. This is illustrated in Figure 34 which shows the phase response of the transverse tissue potentials to oscillations in I.A.A. concentration for two typical plants.

In Figure 35 the amplitude and phase responses evoked by oscillations in I.A.A. concentration are shown for typical plants on an harmonic vector response diagram. The change in phase by two right angles and the rapid phase change with period near the resonance are again the main features.

FIGURE 33

The relations between the double amplitude (a) and the phase (b) responses of the extracellular potentials at the elongating regions and the period of oscillation of I.A.A. concentration (0 to 10^{-7}M) for two plants. The natural periods of oscillation for the two plants are shown on the period axis of (a).

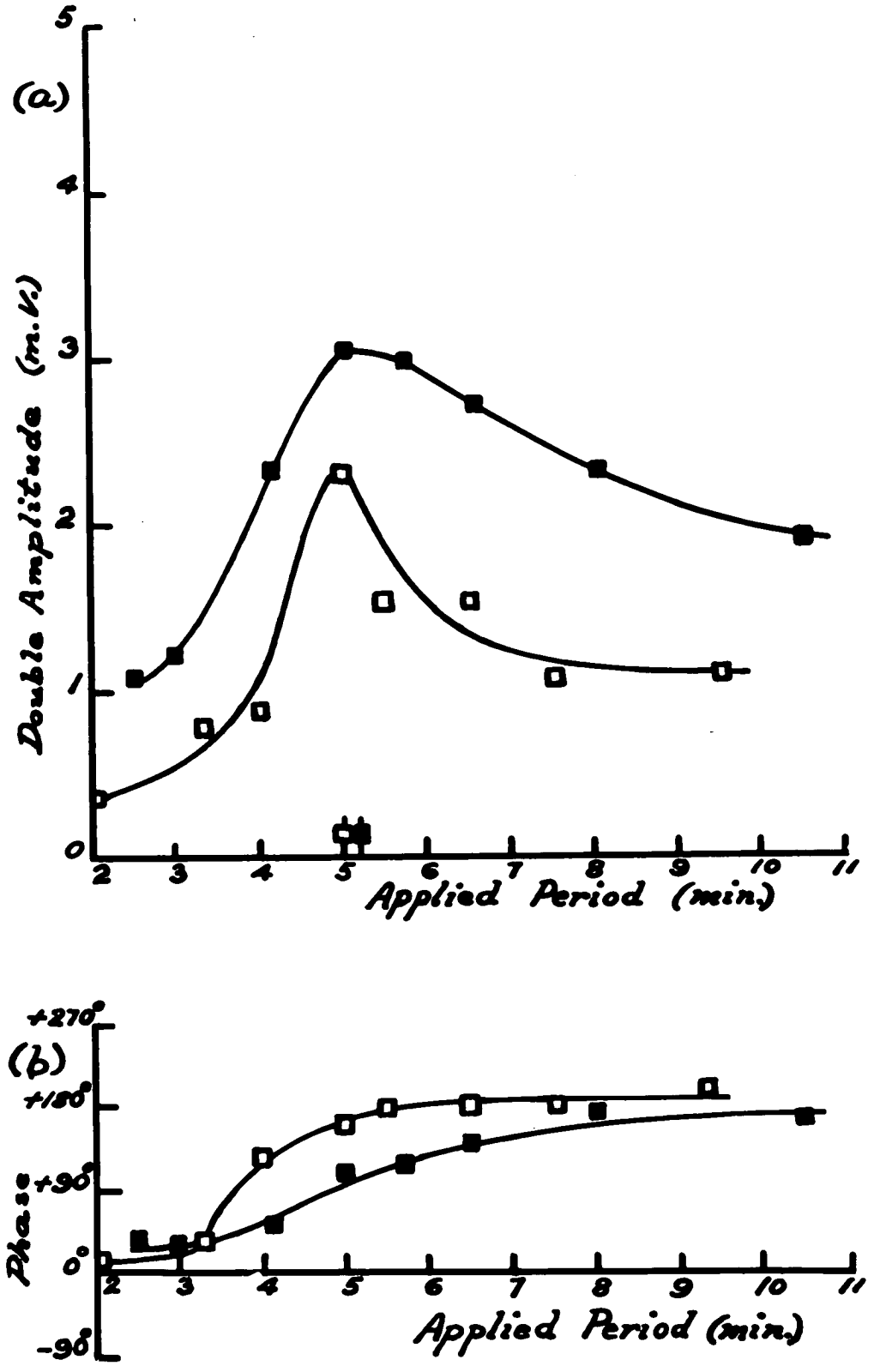
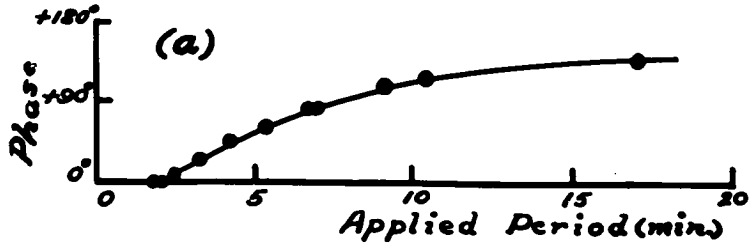

(a)
Double Amplitude (m.k)
0
1
2
3
4
5
2
3
4
5
6
7
8
9
10
11
Applied Period (min.)
(b)
Phase
+270°
+180°
+90°
0°
-90°
2
3
4
5
6
7
8
9
10
11
Applied Period (min.)

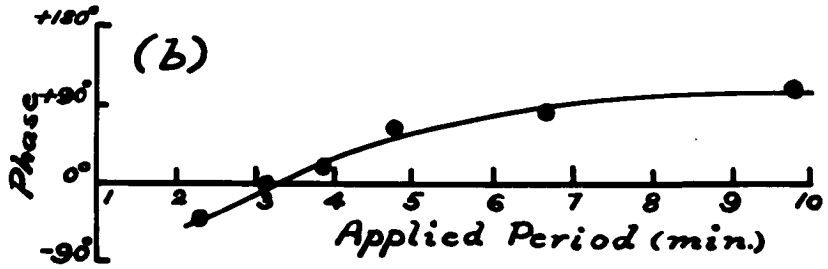
FIGURE 34

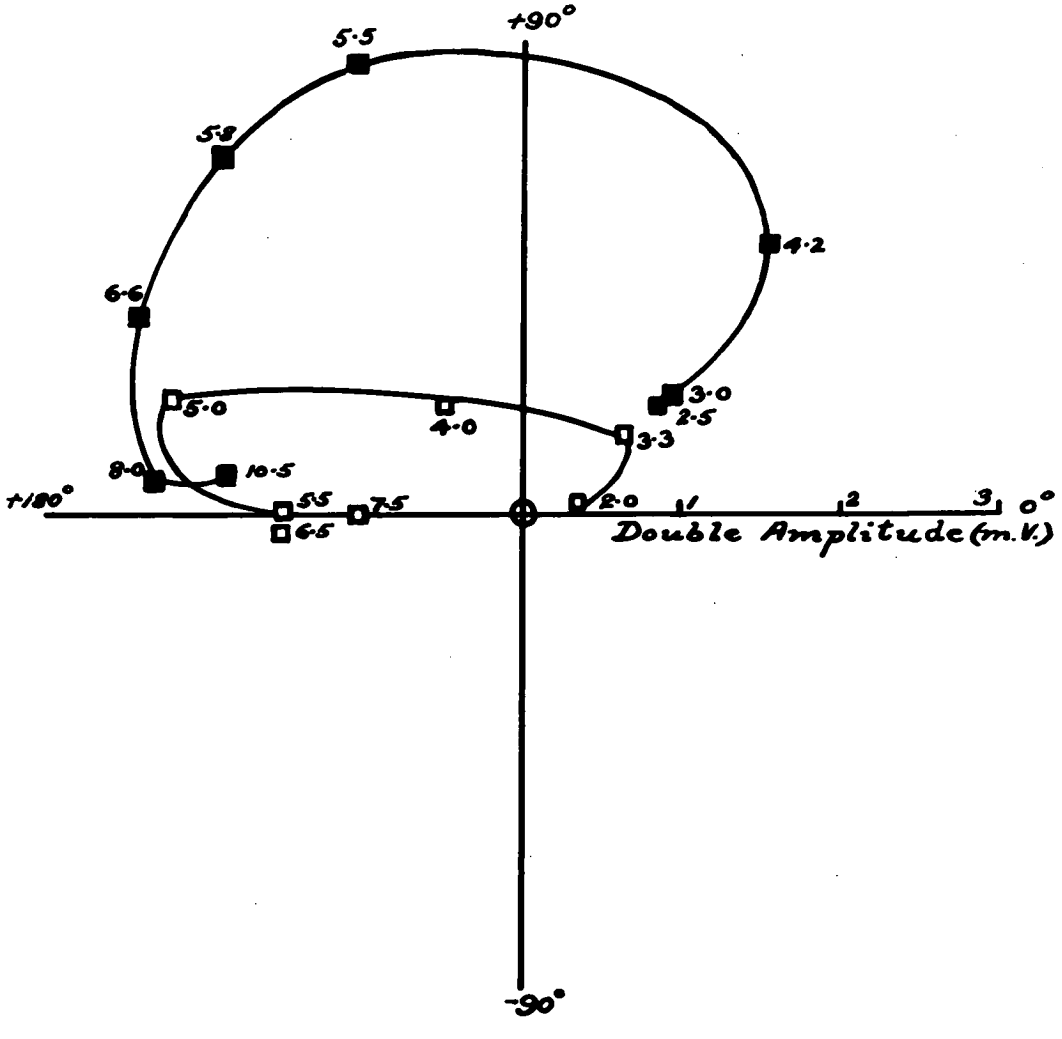
The relations between the phase responses of the transverse tissue potentials and the period of oscillation of I.A.A. solution concentration (0 to 10^{-7}M) for two plants. The potentials were measured at depths of (a) 100μ and (b) 150μ, both at 5 mm. along the root from the tip.

FIGURE 35

Harmonic vector response diagram of the double amplitude and phase responses of the extracellular potentials at the elongating regions of two plants to oscillations in I.A.A. solution concentration (0 to 10^{-7}M) at the periods (in minutes) shown beside the points plotted.


(a)
+180°
+90°
0°
Phase
0
5
10
15
20
Applied Period (min.)


(b)
+180°
+90°
0°
-90°
Phase
1
2
3
4
5
6
7
8
9
10
Applied Period (min.)

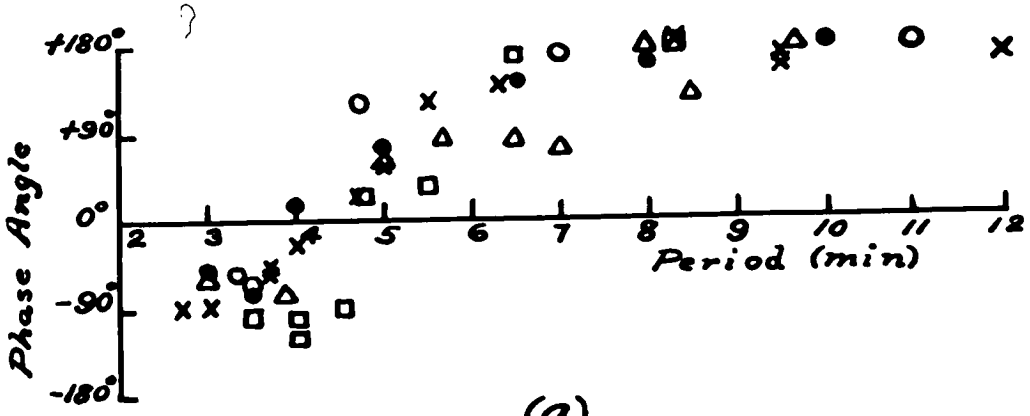

+90°
5·5
5·8
4·2
6·6
5·0
4·0
3·0
2·5
3·3
8·0
10·5
+180°
5·5
7·5
6·5
2·0
1
2
3
0°
Double Amplitude (m.V.)
-90°

In Section III, 5, Figure 28, it was seen that the phase of the forced potential oscillations varies from point to point along the plant root, the oscillations produced at the elongating region being substantially , though not exactly, in antiphase with those observed at the more passive regions. Hence if phase response curves such as those in Figure 31(b) were drawn for the potential responses at the passive regions, these curves would be displaced by about 180° with respect to those in Figure 31(b) for the elongating region. The phase curves for the passive regions, however, still show the same phase change (about 270°) from short to long period. In some cases even the phase curves for the elongating regions of different roots are displaced somewhat with respect to one another although each still shows the same phase change (about 270°) from short to long period. The curves in Figure 31(b) however are the most common type for the elongating or actively resonant region. Consequently to clarify the comparison between phase curves for a number of different plants, they have been normalised so that the phase angle at long periods is +180°. This comparison of such normalised data is shown in Figure 36(a), different symbols being used to denote different plants.

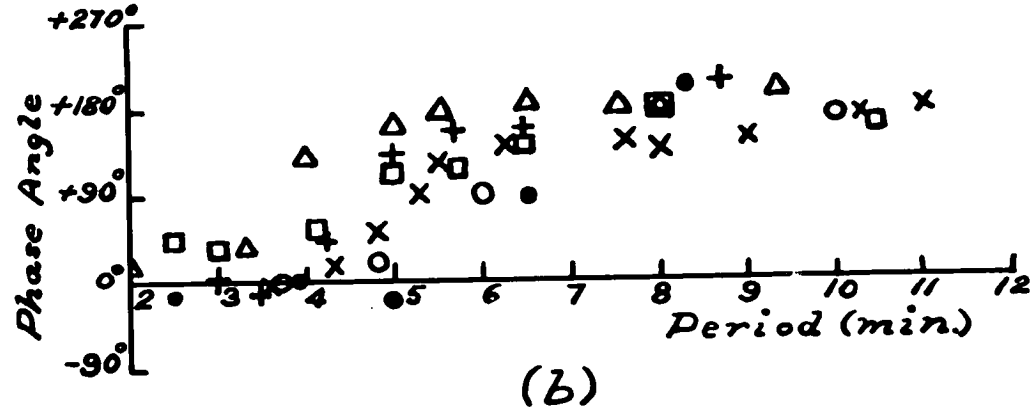
Figure 36(b) shows the phase results for the extracellular potential responses for a number of plants to I.A.A. concentration oscillations. Again the phase angles for each plant have been

FIGURE 36

The relations between the normalised (see text p.53) phase responses of the extracellular potentials and the period of oscillation of (a) osmotic pressure (0 to M/30 sucrose solution) and (b) I.A.A. solution concentration (0 to 10^{-7}M) at the elongating regions of a number of plants (represented by different symbols).


+180°
+90°
0°
-90°
-180°
Phase Angle
2
3
4
5
6
7
8
9
10
11
12
Period (min)

(a)


+270°
+180°
+90°
0°
-90°
Phase Angle
2
3
4
5
6
7
8
9
10
11
12
Period (min.)

(b)

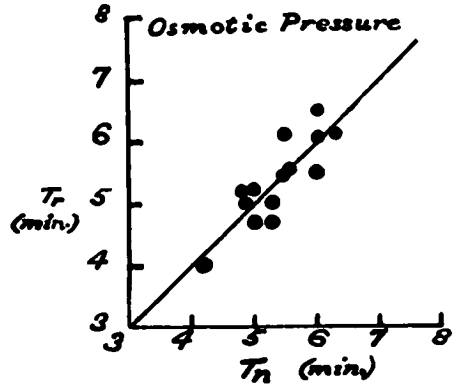
normalised so that the long period phase responses are the same, viz. +180°. Figure 36 again shows the contrast between the phase responses to oscillations in osmotic pressure and I.A.A. concentration.

The close correlation between the natural period of oscillation, obtained from transient potentials, and the resonant period has been mentioned previously in this section. In Figure 37 the resonant periods T_r are shown plotted against the natural periods T_n, each point representing one plant. In Figure 37(a) the resonances were evoked in the extracellular potentials by oscillating the osmotic pressure while in Figure 37(b), by oscillating the I.A.A. concentration. There is a fairly close one to one correlation between the resonant and natural periods in both cases.

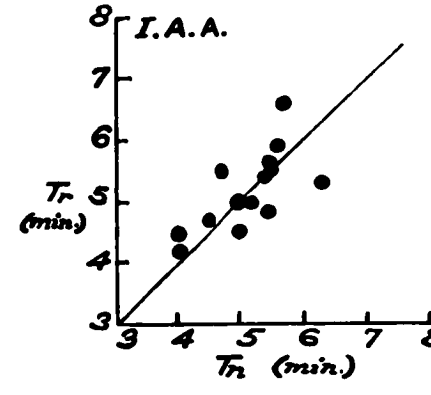
For both intracellular and extracellular potentials there is considerable variability both in the sharpness of the resonances for different plants and in the damping of the transient oscillations of the natural period. For some plants there is no appreciable resonance even at the elongating region of the root. These same plants usually do not exhibit oscillations in their transient potentials or at best, the oscillations are heavily damped. Approximate logarithmic decrements as high as 0.9 have been obtained for these transient oscillations. At the other extreme, some plants exhibit amplitude responses up to five times

FIGURE 37

The relation between the resonant period (Tr) of the extracellular potential and the natural period of potential oscillation. In (a) the potential oscillations were evoked by oscillating the osmotic pressure (0 to M/30 sucrose solution) while in (b) by oscillating the I.A.A. solution concentration (0 to 10^{-7}M).


Osmotic Pressure
T_r (min.)
T_n (min)
8
7
6
5
4
3
3
4
5
6
7
8

(a)


I.A.A.
T_r (min.)
T_n (min.)
8
7
6
5
4
3
3
4
5
6
7
8

(b)

greater at the resonant or natural period than they do at short and long periods. The transient potentials of these plants often show marked oscillations which are only very lightly damped, and in some cases spontaneous oscillations appear. The logarithmic decrements in such cases are negligibly small.

7. The Effect of Replacing Potassium by Calcium

Some limited experiments were undertaken to investigate the effects of different ion species in the plant's bathing solution, on the oscillatory bioelectric potentials. From such an investigation it might be expected that knowledge would be gained regarding specific ions carrying the oscillatory bioelectric currents, or specific ions involved in the physiological system responsible for the potential oscillations. In particular, it is of considerable interest to determine what effect replacement of potassium by a divalent cation, such as calcium, has on the amplitude and phase responses of the plant potentials to applied oscillations in osmotic pressure and auxin concentration. This interest arises from the fact that the effect of auxin on cell walls is intimately connected with the presence of monovalent or divalent cations in the pectic components of cell walls. (Tagawa and Bonner, 1957; Adamson and Adamson, 1958; Van Overbeek, 1959).

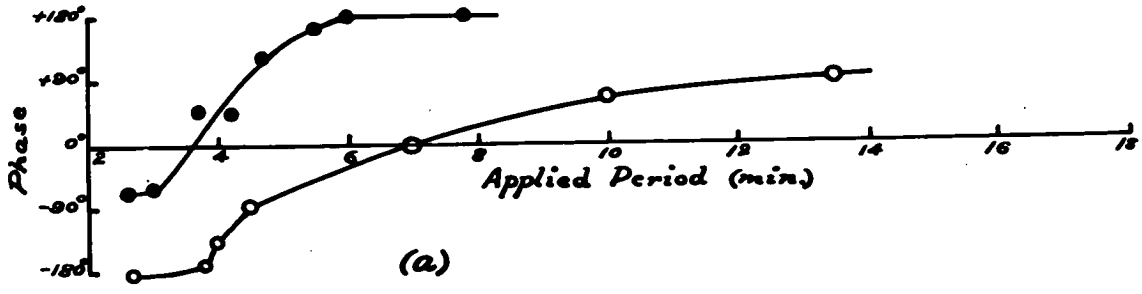
In all the experiments described so far a solution of 10^{-4}M KCl was used as the root's bathing medium, the oscillations in sucrose or mannitol concentration (for osmotic pressure) and I.A.A. concentration being effectively superimposed on the constant background 10^{-4}M KCl solution. In some cases the plant was allowed to equilibrate in this bathing solution for fifteen hours or more before the commencement of experiments. The phase and amplitude responses of these plants to oscillations in osmotic pressure and I.A.A. concentration were the same as those for plants which were allowed to equilibrate for only about one hour after removal from the tap-water culture medium. This suggests that the bioelectric responses described are independent of the presence or absence of various ion species left in the root tissue, originally obtained from the tap-water culture medium. However, it is possible that in both cases (short and long equilibration time in 10^{-4}M KCl) a variety of ion species from the cotyledon material were available to the root throughout the experiments.

In some series of experiments plants were first equilibrated in 10^{-4}M $CaCl_2$ for fifteen hours or more before the oscillations in osmotic pressure or I.A.A. concentration were applied, the background solution still being 10^{-4}M $CaCl_2$. The amplitude and phase responses to osmotic pressure and I.A.A. oscillations were the same under these conditions (Figure 38) as those already described in which a

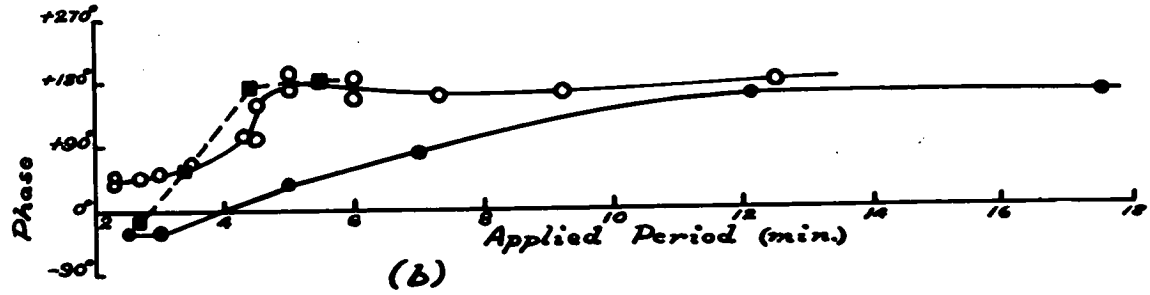
FIGURE 38

The relation between the phase responses of the extracellular potentials at the elongating region and the period of oscillation of (a) osmotic pressure (0 to M/30) and (b) I.A.A. solution concentration (0 to 10^{-7}M).

In both cases the background bathing solution was 10^{-4}M $CaCl_2$ instead of 10^{-4}M KCl as in all previous experiments of this type.


+180°
+90°
0°
-90°
-180°
Phase
2
4
6
8
10
12
14
16
18
Applied Period (min.)

(a)


+270°
+180°
+90°
0°
-90°
Phase
2
4
6
8
10
12
14
16
18
Applied Period (min.)

(b)

background bathing solution of 10^{-4}M KCl was used throughout.

These results suggest that the oscillatory bioelectric currents and the physiological system responsible for the potential oscillations are not dependent on whether the cation in the bathing solution or the root tissue, is monovalent or divalent. This fact will be discussed later in more detail. The effect of different anions has not been studied.

8. Discussion of a Theoretical Feedback Loop Oscillator

It is now proposed to show that the amplitude and phase relations of the bioelectric potentials with respect to the applied oscillations in osmotic pressure or auxin concentration may be explained in terms of a closed feedback loop connecting the bioelectric field to other physiological variables. Very frequently in physiological systems, a sudden change in one variable causes another variable to change to a new value in an exponential manner. The time constant τ of this exponential change is termed the exponential time delay. In the introduction to this section it was found that such inherent time delays acting between physiological variables in a feedback system can result in oscillatory behaviour. Scott (1957) suggested that the simplest closed feedback loop

FIGURE 39

(a) Theoretical feedback loop with three exponential time delays τ_1, τ_2 and τ_3. The associated amplification factors are k_1, k_2 and k_3.

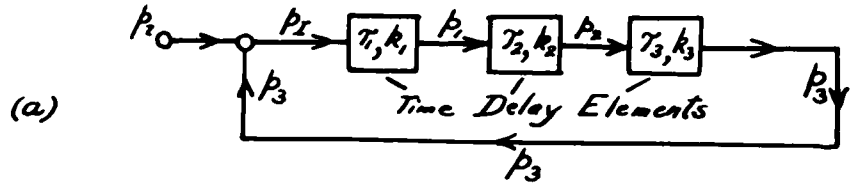
(b) and (c) The relation between the amplitude and phase responses of the signals p_I, p_1, p_2 and p_3 (with respect to the input p_1 of unit amplitude and period T) to the ratio T/τ, where $\tau = \tau_1 = \tau_2 = \tau_3$ in (a).

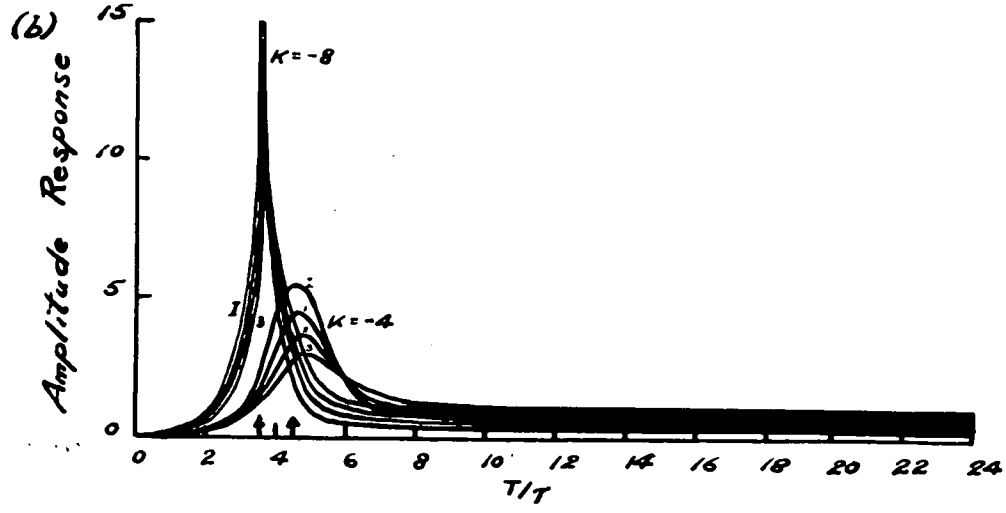
These relations are shown for the cases in which $K = k_1 k_2 k_3 = -4$ and -8. The appropriate natural periods of oscillation for the feedback loop (a) are indicated by arrows on the T/τ axis in (b).

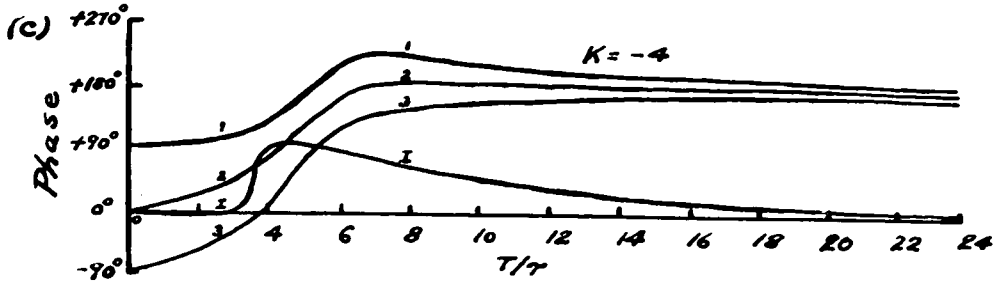
The phase curves in (c) for p_I (i.e. I) and 3 are independent of the individual signs and magnitudes of k_1, k_2 and k_3. They dpend on K only.

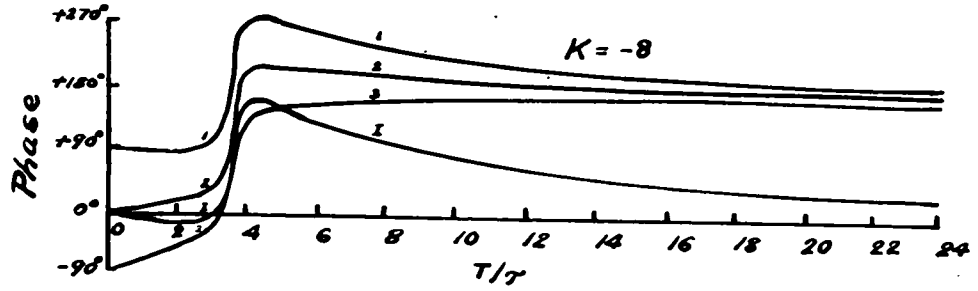
The phase curves for p_1 (i.e. 1) are shown for $k_1 = -\sqrt[3]{4}$ and $-\sqrt[3]{8}$. If k_1 is positive in sign the phase curves are displaced by 180°.

The phase curves for p_2 (i.e. 2) are shown for k_1 and k_2 of opposite sign. If k_1 and k_2 are of the same sign (either + or −) then the phase curves are displaced by 180°.


(a)
p_2
p_1
τ_1, k_1
p_1
τ_2, k_2
p_2
τ_3, k_3
p_3
p_3
p_3
Time Delay Elements


(b)
Amplitude Response
15
10
5
0
K = -8
K = -4
I
0 2 4 6 8 10 12 14 16 18 20 22 24
T/τ


(c)
Phase
+270°
+180°
+90°
0°
-90°
K = -4
I
0 3 4 6 8 10 12 14 16 18 20 22 24
T/τ


Phase
+270°
+180°
+90°
0°
-90°
K = -8
I
0 3 4 6 8 10 12 14 16 18 20 22 24
T/τ

containing only exponential delays between its variables, which can produce sustained oscillations, must contain no less than three delay elements. Such a loop is shown in Figure 39(a).

As well as time delays, other transfer elements are possible. For example amplification or attenuation may be involved. With regard to the time delay element $\mathcal{T}_1, k_1$, (Figure 39(a)) the input variable is p_I and the output p_1. If a sudden change of <u>unit magnitude</u> occurs in p_I, then p_1 will change exponentially (time constant $\mathcal{T}_1$) by a final amount k_1. Thus k_1 is the amplification factor involved in the delay element. The value of k_1 may be greater or less than unity and its sign may be positive or negative. If the sign of k_1 is negative an increase in p_I will cause a decrease in p_1.

For the input and output variables of the delay stages in Figure 39(a) the following formal relations obtain:

$$p_1 + \mathcal{T}_1 \frac{dp_1}{dt} = k_1 p_I \qquad (1)$$

$$p_2 + \mathcal{T}_2 \frac{dp_2}{dt} = k_2 p_1 \qquad (2)$$

$$p_3 + \tau_3 \frac{dp_3}{dt} = k_3 p_2 \qquad (3)$$

We now consider the introduction of an externally applied disturbance p_i. If $p_i = 0$, then $p_I = p_3$. In general, however,

$$p_I = p_i + p_3 \qquad (4)$$

Equations (1) to (4) characterise the behaviour of the loop variables.

It is now necessary to determine the response of the variables p_I, p_1, p_2, p_3 to an externally applied disturbance p_i of oscillatory form. In the following treatment it is assumed that there is no transient or spontaneous oscillatory behaviour at the loop's characteristic periodicity, i.e. only forced oscillatory responses are considered.

The analysis is simplified if τ_1, τ_2, τ_3 are equal, and still predicts the main features of the system.

If p_i is of sinusoidal form, the terms containing differential coefficients may be rewritten in harmonic vector form. Hence

$$\tau_1 \frac{dp_1}{dt} = j\tau\omega p_1 \qquad (5)$$

$$\mathcal{T}_2 \frac{dp_2}{dt} = j\mathcal{T}\omega\, p_2 \qquad (6)$$

$$\mathcal{T}_3 \frac{dp_3}{dt} = j\mathcal{T}\omega\, p_3 \qquad (7)$$

where $\mathcal{T} = \mathcal{T}_1 = \mathcal{T}_2 = \mathcal{T}_3$ and $j = \sqrt{-1}$ and $\omega = 2\pi/T$, where T is the period of the sinusoidal input p_i.

After substituting (5), (6) and (7) in (1), (2) and (3) respectively, the equations (1) to (4) may be solved for p_I/p_i, p_1/p_i, p_2/p_i and p_3/p_i. The expressions for these quantities are in the complex form $a + jb$. For example

$$p_I/p_i = a_I + jb_I$$

Then the amplitude of p_I produced by unit amplitude p_i is given by

$$A_{Ii} = \sqrt{a_I^2 + b_I^2}$$

and the phase of p_I with respect to p_i is given by $\phi_{Ii} = \tan^{-1} b_I/a_I$. Similarly for p_1/p_i, p_2/p_i and p_3/p_i.

These solutions for a_I, a_1, a_2, a_3 and b_I, b_1, b_2, b_3 are as follows:

$$a_I = (1 - K + 3(1 + K)\tau^2\omega^2 + 3\tau^4\omega^4 + \tau^6\omega^6) / C \quad (8)$$

$$a_1 = (k_1\{(1 - K) + (2 + K)\tau^2\omega^2 + \tau^4\omega^4\}) / C \quad (9)$$

$$a_2 = k_1k_2(1 - K - \tau^4\omega^4) / C \quad (10)$$

$$a_3 = K(1 - K - 3\tau^2\omega^2) / C \quad (11)$$

$$b_I = K(\tau^3\omega^3 - 3\tau\omega) / C \quad (12)$$

$$b_1 = (-k_1\{(1 + 2K)\tau\omega + 2\tau^3\omega^3 + \tau^5\omega^5\}) / C \quad (13)$$

$$b_2 = (-k_1k_2\{(K + 2)\tau\omega + 2\tau^3\omega^3\} / C \quad (14)$$

$$b_3 = K(\tau^3\omega^3 - 3\tau\omega) / C \quad (15)$$

where $C = (1 - K - 3\tau^2\omega^2)^2 + (3\tau\omega - \tau^3\omega^3)^2$

In Figures 39(b) and (c) the amplitude and phase responses (obtained from equations (8) to (15)) are shown plotted against T/τ for the cases in which $K(=k_1k_2k_3) = -8$ and -4.

The significance of the loop amplification factor K may be seen from the following discussion. From equations (1) to (3), if $p_i = 0$ (i.e. no input signal), we may solve for any of the three variables p_1, p_2 or p_3. The resulting equation is given in terms of p where p_1, p_2 or p_3 may be substituted for p.

$$\tau_1\tau_2\tau_3 \frac{d^3p}{dt^3} + (\tau_1\tau_2 + \tau_2\tau_3 + \tau_3\tau_1) \frac{d^2p}{dt^2} + (\tau_1 + \tau_2 + \tau_3) \frac{dp}{dt} + (1 - K)p = 0 \qquad (16)$$

If as previously $\tau_1 = \tau_2 = \tau_3 = \tau$, this equation becomes

$$\tau^3 \frac{d^3p}{dt^3} + 3\tau^2 \frac{d^2p}{dt^2} + 3\tau \frac{dp}{dt} + (1 - K)p = 0$$

which yields a solution of the form

$$p = p_o e^{+\alpha t/\tau} + p_1 e^{+\beta t/\tau} \cos\left(\frac{2\pi t}{T_n}\right) \qquad (17)$$

where

$$T_n = \frac{2\pi\tau}{\sqrt{\gamma^2 - \beta^2}} \qquad (18)$$

and

$$-\alpha\gamma^2 = 1 - k_1k_2k_3 = 1 - K \qquad (19)$$

$$-\alpha - 2\beta = 3 \qquad (20)$$

$$\gamma^2 + 2\alpha\beta = 3 \qquad (21)$$

Equation (17) contains an exponential term and an oscillatory term for which the damping coefficient (logarithmic decrement) is given by $-\beta T_n/\tau$ where T_n is the natural period of oscillation of the system. The relation between K and the logarithmic decrement is shown in Figure 40. When $\beta = 0$ (the logarithmic decrement is also equal to zero) there is no damping and $K = -8$. At this value of K the system can oscillate spontaneously. For $\beta = -0.2$, $K = -4$, the logarithmic decrement is approximately 0.9, i.e. the oscillation is heavily damped.

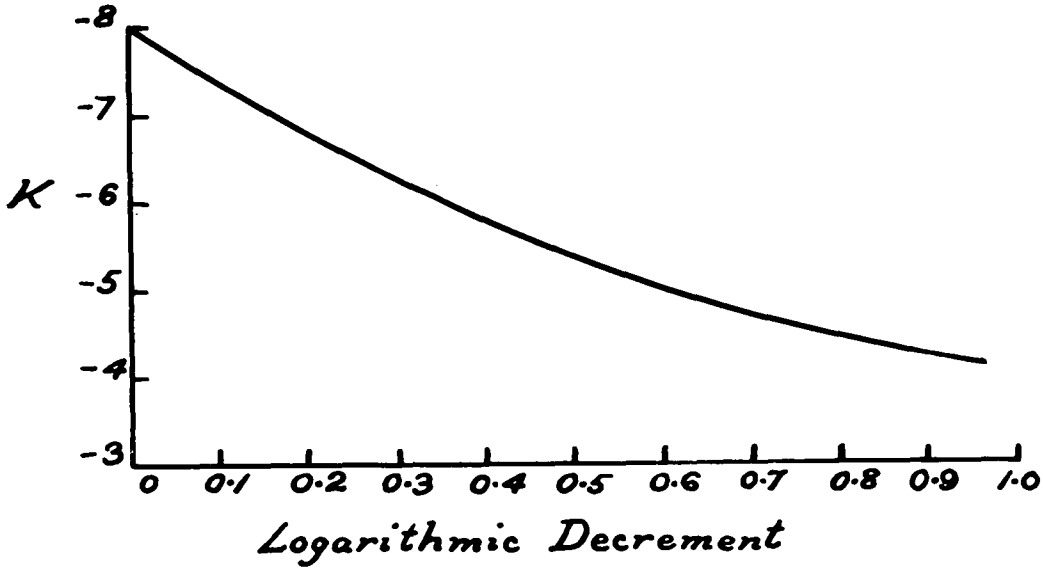
The negative values of K indicate that for an input oscillation of very long period or a very slow change, the feedback signal is in antiphase so as to oppose the input disturbance. The system then has negative feedback and acts as a control system for its variables.

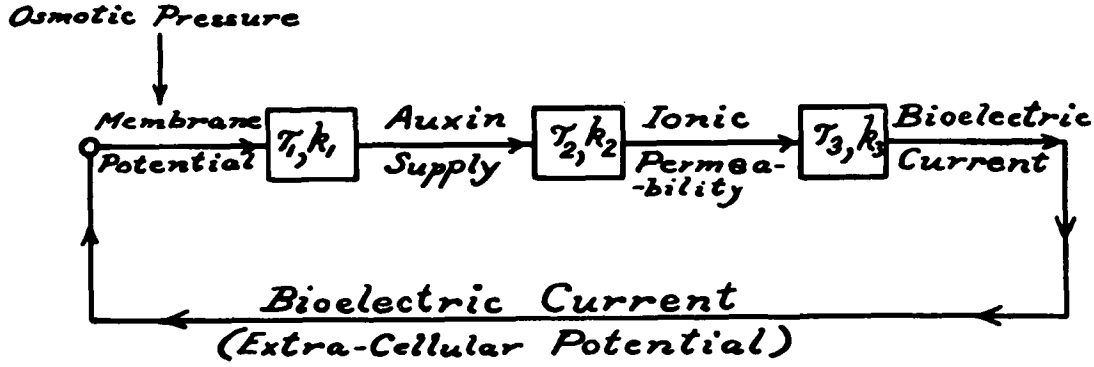
FIGURE 40

The relation between $K(=k_1k_2k_3)$ and the logarithmic decrement (damping coefficient) of natural oscillations in the feedback loop of Figure 39(a) when $\tau_1 = \tau_2 = \tau_3 = \tau$, and the feedback is negative.

FIGURE 41

A physiological feedback loop with three exponential delays. This negative feedback loop of control is consistent with both the theoretical loop of Figure 39(a) and the physicological phenomena associated with the bioelectric oscillations of bean roots.


-8
-7
K -6
-5
-4
-3
0 0·1 0·2 0·3 0·4 0·5 0·6 0·7 0·8 0·9 1·0
Logarithmic Decrement


Osmotic Pressure
Membrane Potential
T_1, k_1
Auxin Supply
T_2, k_2
Ionic Permea-bility
T_3, k_3
Bioelectric Current
Bioelectric Current
(Extra-Cellular Potential)

From Figures 39(b) and (c) it is apparent that the general features of the phase and amplitude relations are similar in the two cases ($K = -4$ and -8). The main features are resonances in all variables, total phase changes from short to long period of 0, 1, 2 and 3 right angles for the variables p_I, p_1, p_2 and p_3 respectively relative to p_i. It will be seen by comparing Figures 39(a) and 39(c) that the total phase change in right angles from short to long period for p (whether p_I, p_1, p_2 or p_3) is given by the number of delays between p_i and p counting in the forward direction of the arrows around the loop.

From equations (18) to (21), T_n/τ may be calculated. In Figure 39(b) it is seen that for $K = -8$, $T_n/\tau = 3.5$ while for $K = -4$, $T_n/\tau = 4.5$. Since the natural period (T_n) of the bioelectric oscillations is between 4 and 7 minutes (Section III,6, Figure 37), the time delays (τ) must be approximately 1 or 2 minutes if $\tau_1 = \tau_2 = \tau_3 = \tau$. However, τ_1, τ_2, τ_3 need not necessarily be equal, as assumed for simplibity. For instance, two of the delays may be considerably greater than 1 or 2 minutes while the third would be considerably less. Alternatively two of the delays may be less and the other greater. Then again one delay may be greater, one less and one approximately equal to 1 or 2 minutes.

However, it may readily be shown by substituting appropriate nominal values for the three time delays τ_i (τ_1, τ_2 and τ_3) in equation (16) and solving for T_n/τ_i, that for any one of the time delays τ_1, the value of T_n/τ_i does not diverge from 4 by more than a power of ten. In other words for a given value of T_n, if any one of the time constants τ_i diverge from $T_n/4$ by more than a power of ten, then it ceases to be an effective time delay in the feedback loop responsible for the oscillations of period T_n.

The results described in this section may now be compared with the predictions of the above feedback loop theory. It was found that there is no change in the phase angle of the transverse tissue potentials with respect to the applied oscillations in osmotic pressure as the period is changed from short to long periods. This means there is no appreciable delay between the osmotic pressure variation and the resulting variation in the transverse tissue potential. This is to be expected since a sudden change in osmotic pressure caused a rapid change in the intracellular potentials, the delay involved being only a few seconds (Section III,3). This delay is negligible compared with the periods of the applied oscillations in osmotic pressure.

The phase change from short to long periods of three right angles for the extracellular potentials with respect to the applied osmotic pressure oscillations suggests that there are three

effective time delays between the osmotic pressure variation and the resulting variation in the extracellular potential. For the corresponding case of the extracellular potentials and the I.A.A. concentration oscillation the phase changes of two right angles suggests that there are two delays between the oscillatory I.A.A. concentration and the potential response, both intracellular and extracellular.

9. Discussion of a Proposed Physiological Feedback Loop

Figure 41 shows a physiological feedback loop, based on the formal model discussed above, which is consistent with the results described. In this loop a change in osmotic pressure causes an immediate change (negligible delay) in the membrane potentials of the root's outer cells (i.e. the epidermal and outer cortical cells). This is in accord with the fact that following a sudden osmotic pressure change the intracellular potentials (either transverse tissue potentials or the intracellular potentials with respect to a distant reference) change to a new value within a few seconds, which is negligible with respect to the periods of oscillation considered. This fast component in the potential change was considered to arise from the movement of water into or out of the root cells following a change in osmotic pressure of the plant's bathing solution.

Negligible delay in such a process is to be expected because of the high permeability of plant cell membranes to water (Dainty and Hope, 1959). This high water permeability is evidenced also by the fact that plasmolysis, which is caused by the efflux of water from a cell, occurs within seconds of the cell's bathing solution reaching an osmotic pressure greater than that of the cell sap.

When the water content of a cell is changed the ionic concentration of its interior changes also. This in turn determines the potential across the cell membrane separating the interior of the cell from the outside. It is the sum of these membrane potentials across the tissue which determines the transverse tissue potentials.

The electric fields across the cell membranes are then considered as affecting the supply of auxin to the cell walls, the cytoplasm and also to the membranes themselves. Following a change in the membrane potential it is envisaged that there is a time delay τ_1 before the resulting change in auxin supply occurs. There is a very considerable body of evidence for the transport of auxin by means of bioelectric fields. Much of this evidence has been accumulated in the bioelectric study of various tropisms including phototropism, geotropism, electrotropism and the growth response following mechanical stimulation in the Avena coleoptile and other plant organs. (Brauner and Bunning 1930, Amlong 1933, Koch 1934,

Schrank 1945, 1946, 1957, Schrank and Backus 1951, Webster and Schrank 1953, Wiegand and Schrank 1954).

Moreover, it has been shown that for all these tropisms in coleoptiles, a change in the bioelectric field always preceeds the growth response. (Schrank 1945, 1946, 1951; Backus and Schrank, 1951). The correlations between the bioelectric response and the tropic response suggest that auxin is transported electrophoretically by the bioelectric field. It is possible however that the change in auxin distribution following tropic stimulation is not caused merely by transport but by an asymmetric redistribution of the total auxin content of the coleoptile. This could arise from destruction or inhibited production of auxin on one side of the coleoptile or enhanced production on the other. In phototropic responses these mechanisms do not seem tenable, for in zea maize coleoptiles the total auxin content (the sum of both sides) remains the same both before and after unilateral illumination even though in the latter there is much more auxin on the dark side than on the light side. It seems unlikely that auxin would be destroyed, or its production inhibited, on the light side, and its production enhanced on the dark side so that the decrease in the former and the increase in the latter would be almost equal. Lateral auxin transport from the light side to the dark side appears a more likely mechanism (Briggs, Tocher and Wilson, 1957).

The suggestion that auxin is transported by the bioelectric field has been criticised on the grounds that these fields are not sufficiently strong. Clark (1937) has shown that there is no electrophoretic transport of I.A.A. through agar blocks unless the applied fields are greater than 50V/cm. The average bioelectric fields across bulk tissue are less than this value by several orders of magnitude. However, the fields across biological membranes are much larger.

The potentials across the membranes of plant cells are often of the order of several millivolts. This was shown for the epidermal and outer cortical cells of bean roots in Section II, 5. For single celled Algae such as Nitella the membrane potentials are of the order of 100 m.v. (Walker 1955, Findlay 1959). Various estimates of cellmembrane thickness in plant and animal tissue have been made from resistance and capacitance data. These estimates have been substantially confirmed by electron micrograph measurements. The thicknesses of a considerable anatomical variety of synaptic membranes for various animals have been summarised by Eccles and Jaeger (1958). The thickness values all lie between 50 and 100 $\mathring{A}$. From electron micrograph studies, the membrane thickness of chloroplasts in Nitella is found to be 70 $\mathring{A}$ (Mercer, Hodge, Hope and McLean, 1955).

If the membrane thickness be taken as 100 Å and the membrane potential 1 m.V. then the associated electric field is 10^6 V/cm. Hence the observed membrane potentials are quite sufficient to cause electrophoretic transport of auxin from one side of a membrane to the other. Hence the supply and distribution of auxin at membranes and the neighbouring cell walls could be affected very considerably and fairly quickly (time delay τ_1) by changes in the membrane potentials. In this way auxin could be transferred from cell to cell through the tissue even though the average bioelectric field across the plant tissue in bulk is insufficient for electrophoretic transport.

In a considerable variety of tissues the effect of auxin on membrane permeability to water, ions and other solutes has been studied. The sensitivity of membrane permeability to auxin in such divers plant tissues as bean endocarp, the abscission zone of Coleus and the leaves of Mesembryanthemum sp. and Rhoeo discolor has been reported by Sacher (1957, 1959) and by Sacher and Glasziou (1959). Ling and Gerard (1949) showed that I.A.A. at 2.5×10^{-4}M and 5×10^{-3}M increases the permeability to potassium in the Rana pipiens sartorius fibre membrane. The effect is reversible on removal of the I.A.A. Bennet-Clark (1955) has suggested that the effect of auxin on the ionic permeability of plant cell membranes

is exerted through an acetylcholine mediated system.

The change in ionic permeability of plant cell membranes, effected by a change in auxin concentration or supply, may be caused primarily by auxin induced cell wall plasticity. In decapitated Avena coleoptiles this cell wall plasticization occurs within a few minutes after the auxin addition (Adamson, personal communication). This in turn permits the osmotic uptake of water by the cell so stretching the plastic cell wall and the cell membrane enclosing the protoplasm. (Adamson and Heather Adamson, 1958; Van Overbeek, 1959). This mechanical stretching of the cell membrane could change its permeability to ions and other solutes.

There would of course be a time delay (τ_2) between the change in auxin concentration and the resulting change in membrane permeability. This ionic permeability change alters the ionic transfer across the tissue, so changing the bioelectric current and therefore the extracellular bioelectric field (time delay τ_3 involved). The change in bioelectric current causes a corresponding change in the membrane potentials, i.e. it is "fed back" to the membrane potential. There is no time delay for this process, being a purely electric (ohmic) effect. This then completes the feedback loop.

It will be recalled from Section III, 3, Figures 17 and 18, that a sudden change in osmotic pressure causes a rapid change in the intracellular potentials (both transverse tissue potential and the intracellular potential with respect to a distant reference) but not in the extracellular potential. This fast component was attributed to the non-ohmic potential component across the cell membranes. The slow variation in both the extracellular and intracellular potentials, caused by sudden changes in osmotic pressure or I.A.A. concentration, was attributed to the ohmic potential component both inside and outside the tissue. It is this slow, ohmic potential component which is "fed back" to oppose and correct the initial rapid change in the membrane potential (and therefore in the transverse tissue potential)(Section III, 3, Figure 19).

From Figure 41, it is apparent that between the auxin supply and the bioelectric current (or the extracellular bioelectric field) there are two delays, and between the osmotic pressure and the bioelectric current, three delays. Thus the physiological model is in agreement with the phase and amplitude relations described in this section. It is however probable that other physiological feedback loops could be envisaged which would be equally in accord with the results described but the one discussed above appears to be the simplest. Other more complex feedback loops will be discussed later in this section.

10. Discussion of the Cell Wall Donnan System and its Auxin Sensitivity

The effect of replacing potassium by divalent ions such as calcium or magnesium in the cell walls of both coleoptiles and roots is to render the cell walls more rigid. The effect is reversible in that subsequent replacement of the divalent ions by monovalent ions such as potassium or sodium, softens the walls again. (Tagawa and Bonner, 1957; Van Overbeek, 1959). Van Overbeek suggests that cell walls contain pectic chains cross-linked at intervals by ionic bonds in which calcium ions bind carboxylate ions of adjacent pectic chains. Replacement of calcium by potassium weakens the bonds so plasticising the cell wall. He suggested also that auxin breaks the calcium bonds by methyl estirification again resulting in plasticisation of the cell walls. (Van Overbeek, 1959).

There is very good evidence that these suggestions are correct. It appears that methyl esters, the presence of which render the cell wall plastic, are converted to carboxyl groups by the enzyme pectin methylesterase. When calcium or other divalent ions are present they combine with the carboxyl groups making the pectin chains more rigid and so stiffening the cell wall. Monovalent ions on the other hand do not have this deplasticising effect. Auxins (e.g. I.A.A.) convert the pectin methylesterase to a bound inactive form which does not convert the methyl esters to carboxyls.

(Adamson and Heather Adamson 1958).

The presence of fixed anionic sites in the pectin components of the cell walls, to which cations may be loosely bound, constitutes a Donnan system. This system could conceivably affect the bioelectric potentials of the root by affecting the passage of ions through the cell walls in a manner similar to that of cell membranes. Hence this auxin sensitive ionic system together with, or possibly instead of the cell membranes could constitute the auxin sensitive permeability variable in the feedback loop. This would have been strongly suggested had there been a difference in the type of bioelectric response to applied oscillations in osmotic pressure or auxin concentration when potassium was replaced by calcium in the bathing solution. The fact that there was no qualitative difference for potassium and calcium does not however preclude this possibility.

11. Other Feedback System Elements and Transfer Functions

In practical feedback systems such as mechanical and electrical servomechanisms, many different transfer functions appear in the elements of such systems. Only the exponential time delay element has so far been considered in the proposed bioelectric feedback oscillator. For this element the relation between the input p_i and the output p_o is given by:

$$p_o + \tau \frac{dp_o}{dt} = kp_i$$

This equation may be written in differential operator form such that the operation of differentiation with respect to time $(\frac{d}{dt})$ is replaced by D.

That is

$$p_o + \tau Dp_o = kp_i$$

or

$$(1 + \tau D)p_o = kp_i$$

Hence

$$\frac{p_o}{p_i} = \frac{k}{1 + \tau D}$$

The ratio p_o/p_i is the transfer function of the exponential delay element.

Commonly occurring transfer elements appearing in practical feedback systems are differentiators and integrators. The transfer function for a pure differentiator is

$$p_o/p_i = D$$

since

$$p_o = \frac{dp_i}{dt}$$

For an integrator

$$\frac{p_o}{p_i} = \frac{1}{D}$$

That is

$$\frac{dp_o}{dt} = p_i$$

or

$$p_o = \int p_i \, dt$$

However, in such elements there is usually a constant of proportionality so that the transfer functions for a differentiator and an integrator element may be written

Differentiator	$p_o/p_i = k\tau D$
Integrator	$p_o/p_i = k/\tau D$

Although the significance of including a constant τ in the coefficient of D is not immediately apparent, when a number of such elements, including exponential time delay elements, are combined

in a feedback system, the values of τ in the differentiators and integrators do have some time-constant properties.

In some elements the differentiation or integration of the input p_i is not complete and some of the input appears in the output without being differentiated or integrated. Such elements may be referred to as mixed differentiators and mixed integrators. Their transfer functions are

$$\text{Mixed differentiator} \qquad p_o/p_i = \frac{k}{(1 + 1/\tau D)}$$

$$\text{Mixed integrator} \qquad p_o/p_i = \frac{k}{(1 + \tau D)}$$

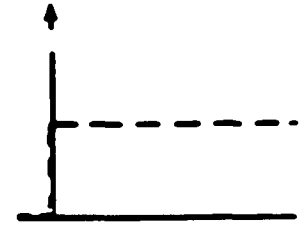
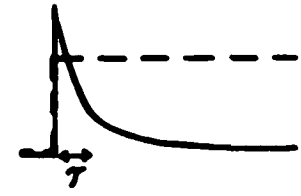
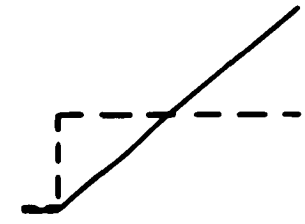
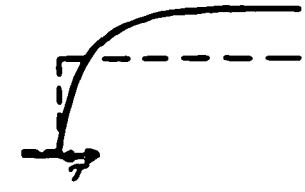
Mixed differentiation frequently occurs in physiological systems, e.g. when a sudden change occurs in the energy output of an animal, the energy made available by phosphate bonds increases very rapidly (almost instantaneously) but very quickly diminishes again as the A.T.P. in the resting metabolic supply is dissipated.

The mixed integration element is seen to be the exponential time delay considered previously.

For step function inputs p_i the output responses p_o for

FIGURE 42

Transfer functions and schematic responses (full lines) to step function inputs (dashed lines) for various transfer elements.

Transfer Element	Transfer Function	Response to Step Function Input
Differentiator	$k\tau D$	
Mixed Differentiator	$k/(1+1/\tau D)$	
Integrator	$k/\tau D$	
Mixed Integrator	$k/(1+\tau D)$	

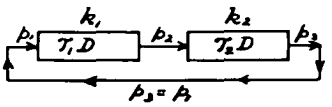
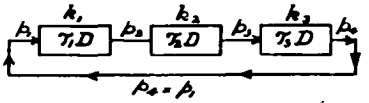
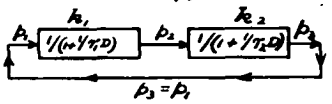
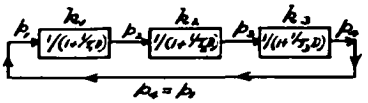
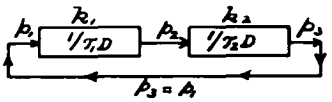
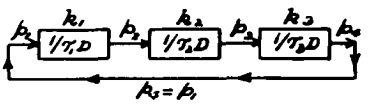
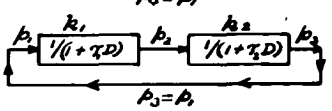
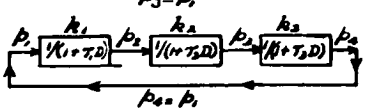
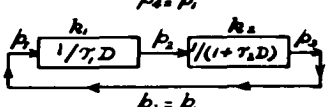
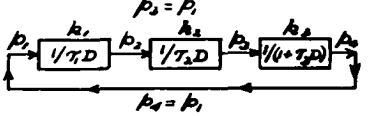
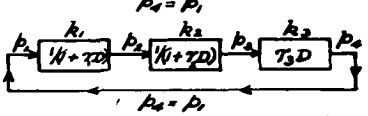
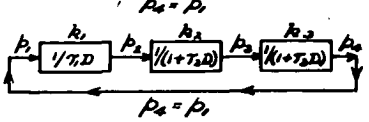
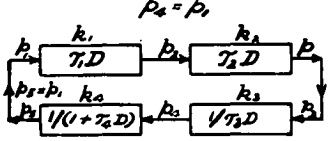
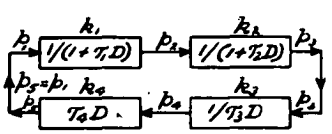
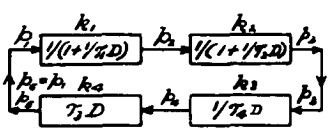
these transfer elements are summarised schematically in Figure 42. If the input be oscillatory, the output from a pure differentiator leads the input by 90° irrespective of the input frequency. For a pure integrator the output lags the input by 90°, the phase lag again being independent of the frequency. For a mixed differentiator or a mixed integrator these phase relations are true only at low and high frequencies respectively, the input and output being in phase at high and low frequencies respectively.

There is of course an infinite number of feedback loops which could be constructed from various combinations of these elements. For sustained oscillations to appear in the variables of a loop it is necessary for the differential equation, describing any variable of the loop, to be of the second order or higher. If the equation be only of the second order then it is also necessary that the coefficient of the first order derivative should vanish. Figure 43 shows a number of different feedback loops constructed from the transfer elements described above. The differential equations describing any of the variables of the loop are tabulated together with the types of oscillatory behaviour of which the loops are capable.

From Figure 43(a) to (e) it is apparent that only two differentiator: or two integrator elements are necessary to construct

FIGURE 43

Various feedback loops, constructed from the transfer elements of Figure 42, together with the differential equations and possible modes of oscillatory behaviour for the variables p (referring to p_1, p_2 .....)

FEEDBACK LOOP	DIFFERENTIAL EQUATION	OSCILLATIONS
(a) 	$\tau_1\tau_2\frac{d^2p}{dt^2} - p = 0$	Sustained
(b) 	$\tau_1\tau_2\tau_3\frac{d^3p}{dt^3} - p = 0$	Sustained or damped
(c) 	$(1-k_1k_2)\tau_1\tau_2\frac{d^2p}{dt^2}+(\tau_1+\tau_2)\frac{dp}{dt}+p=0$	Damped
(d) 	$(1-k_1k_2k_3)\tau_1\tau_2\tau_3\frac{d^3p}{dt^3}+(\tau_1\tau_2+\tau_2\tau_3+\tau_3\tau_1)\frac{d^2p}{dt^2}+(\tau_1+\tau_2+\tau_3)\frac{dp}{dt}+p=0$	Sustained or damped
(e) 	$\tau_1\tau_2\frac{d^2p}{dt^2}-k_1k_2p=0$	Sustained
(f) 	$\tau_1\tau_2\tau_3\frac{d^3p}{dt^3}-k_1k_2k_3p=0$	Sustained or damped
(g) 	$\tau_1\tau_2\frac{d^2p}{dt^2}+(\tau_1+\tau_2)\frac{dp}{dt}+(1-k_1k_2)p=0$	Damped
(h) 	$\tau_1\tau_2\tau_3\frac{d^3p}{dt^3}+(\tau_1\tau_2+\tau_2\tau_3+\tau_3\tau_1)\frac{d^2p}{dt^2}+(\tau_1+\tau_2+\tau_3)\frac{dp}{dt}+(1-k_1k_2k_3)p=0$	Sustained or damped
(i) 	$\tau_1\tau_2\frac{d^2p}{dt^2}+\tau_1\frac{dp}{dt}-k_1k_2p=0$	Damped
(j) 	$\tau_1\tau_2\tau_3\frac{d^3p}{dt^3}+\tau_1\tau_2\frac{d^2p}{dt^2}-k_1k_2k_3p=0$	Sustained or damped
(k) 	$\tau_1\tau_2\frac{d^2p}{dt^2}+(\tau_1+\tau_2-k_1k_2k_3\tau_3)\frac{dp}{dt}+p=0$	Damped
(l) 	$\tau_1\tau_2\tau_3\frac{d^3p}{dt^3}+\tau_1(\tau_2+\tau_3)\frac{d^2p}{dt^2}+\tau_1\frac{dp}{dt}-k_1k_2k_3p=0$	Sustained or damped
(m) 	$(k_1k_2k_3k_4\tau_1\tau_2-\tau_3\tau_4)\frac{d^2p}{dt^2}-\tau_3\frac{dp}{dt}=0$	Damped
(n) 	$\tau_1\tau_2\tau_3\frac{d^3p}{dt^3}+(\tau_1\tau_2+\tau_2\tau_3+\tau_3\tau_1)\frac{d^2p}{dt^2}+(\tau_3-k_1k_2k_3k_4\tau_4)\frac{dp}{dp}=0$	Sustained or dampea
(o) 	$(k_1k_2k_3k_4\tau_1\tau_2\tau_3-\tau_1\tau_2\tau_4)\frac{d^2p}{dt^2}-(\tau_1+\tau_2)\tau_4\frac{d^3p}{dt^3}-\tau_4\frac{dp}{dt}=0$	Sustained or damped

a loop capable of producing sustained oscillations in its variables. It is unlikely however that pure differentiation or integration would occur in a physiological system. Indeed it is seldom that pure differentiation or integration at all frequencies can be achieved even in specially designed practical systems. The more probable type of transfer element is that involving mixed differentiation or the exponential time delay type of integration. When either of these elements is present in the loop, at least three transfer elements are necessary for sustained oscillations. This may be seen by comparing (c) and (d), (g) and (h), (i) and (j) in Figure 43. Figures 43(k) and (l) show two loops each with three elements, (l) is capable of producing sustained oscillations while for (k) only damped oscillations are possible.

Figures 43(m), (n) and (o) show loops containing four elements; (m) is capable of producing only damped oscillations while (n) and (o) are capable of sustained oscillations. In a loop where at least one transfer element is of the mixed differentiator or mixed integrator (exponential time delay) type, the differential equation describing the variables contains a first orderderivative the coefficient of which is non-zero, i.e. a damping term. Hence for these cases the necessary condition for sustained oscillations is that the differential equation of the loop variables should be of the third or higher order. The order of the differential

equation is determined by the power of D in the numerator or denominator (whichever power is the higher) of the loop transfer function (i.e. the product of the transfer functions of all the individual transfer elements of the loop). For all possible single loops constructed from the four transfer elements discussed, the differential equation describing the loop variables is the same for all variables in the loop and is independent of the order in which the elements are arranged in the loop.

It is quite possible that transfer elements from one physiological variable to another are not merely exponential time delay elements. Differentiator or integrator elements of the type discussed as well as other functional relations may obtain, which would give rise to the production of oscillations of a characteristic period. Further, various combinations of such elements could yield the phase relations existing between an oscillatory input (such as osmotic pressure and I.A.A. concentration) and the observed bioelectric output response.

Apart from the exponential time delay, another type of delay occurs frequently in the animal nervous system. This is the finite time delay existing between the stimulation of one part of a nerve fibre and the response at some other point on the fibre along which the stimulus is propagated in the form of an action potential.

The time delays at synaptic junctions and the latency of nerve cells are also of this type. These finite delays may however be formally treated, to a fair approximation, as a number of exponential delays in series.

In general, an examination of oscillatory physiological systems to which feedback analysis has been applied, such as those described briefly in Section III, 1, it has been found that the exponential time delay is the most probable transfer element. Consequently it has been used to describe the observed phenomena in the case of bioelectric oscillations produced by bean roots.

12. Multiple Feedback Loops

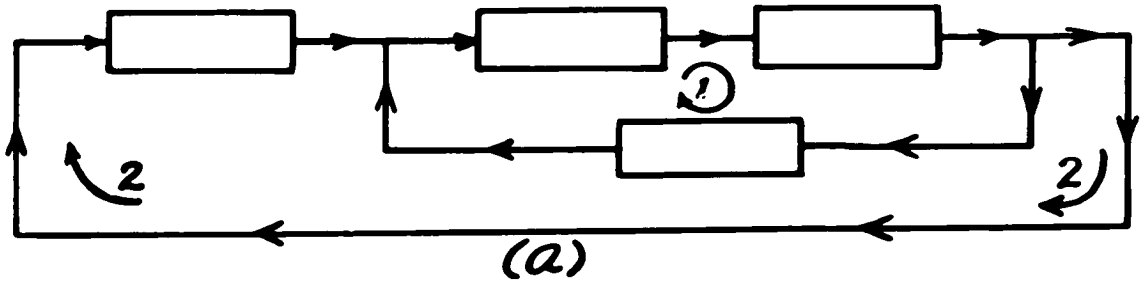
In many practical feedback systems, there is not merely a single closed loop but two or more loops linked together to form a multiple loop. A double loop is shown in Figure 44(a) containing two sub-loops (1) and (2). In this loop and other multiple loops to be discussed, only exponential time delay elements will be considered. Both sub-loops (1) and (2) contain three delay elements so that each, in itself, could produce sustained oscillations of its own characteristic frequency. Hence there are two possible modes of oscillation for the double loop. Figure 44(b) shows another double loop, each sub-loop of which again contains three delay elements, and consequently the double loop is again capable of two

FIGURE 44

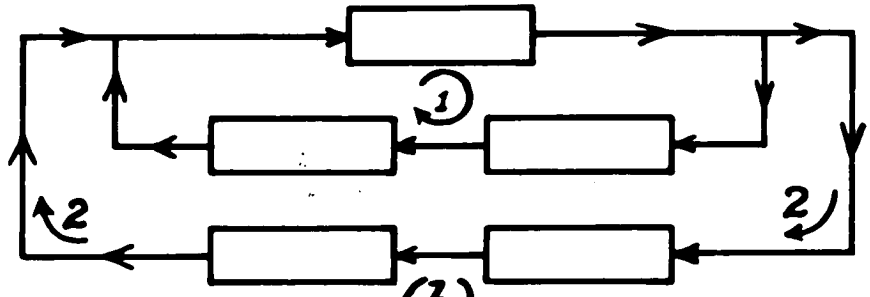
Three double feedback loops containing only exponential delay transfer elements.

(a) and (b) Three delays in each sub-loop.

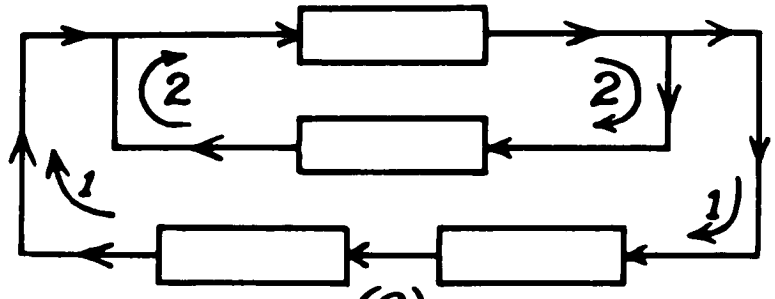
(c) Sub-loop (1) has three delays, sub-loop (2) has only two delays.



(a)



(b)



(c)

modes of oscillation.

The loop in Figure 44(c) consists of a three element sub-loop (1) and a two element sub-loop (2). The latter can have only a damped oscillatory mode while sub-loop (1) is capable of sustained oscillations. If sustained oscillations appear in sub-loop (1) at its characteristic frequency, sub-loop (2) might well be energised to oscillate at its characteristic frequency. However, the oscillations in sub-loop (2) would probably be irregular and possibly not sustained.

Although three time delay elements are necessary for a feedback loop to be inherently capable of sustained oscillation, certain conditions relating the time delays (τ) and the amplification factors (k) of the three transfer elements must obtain or else the oscillations would be damped (Sections III, 8, and V, 2). Consequently if a sudden disturbance, such as a step function input, be introduced in multiple loops, such as (a) or (b), it is possible that at least one of the two resulting oscillatory modes in the transient would be damped. For loop (c), only the oscillation in sub-loop (1) could possibly be inherently sustained. The various possibilities for these double loops are shown in Figure 45.

In Section I, 4, it was seen that oscillations of two different periods are occasionally observed in potential transients. Hence

FIGURE 45

Oscillations which could be produced by the double feedback loops of Figure 44.

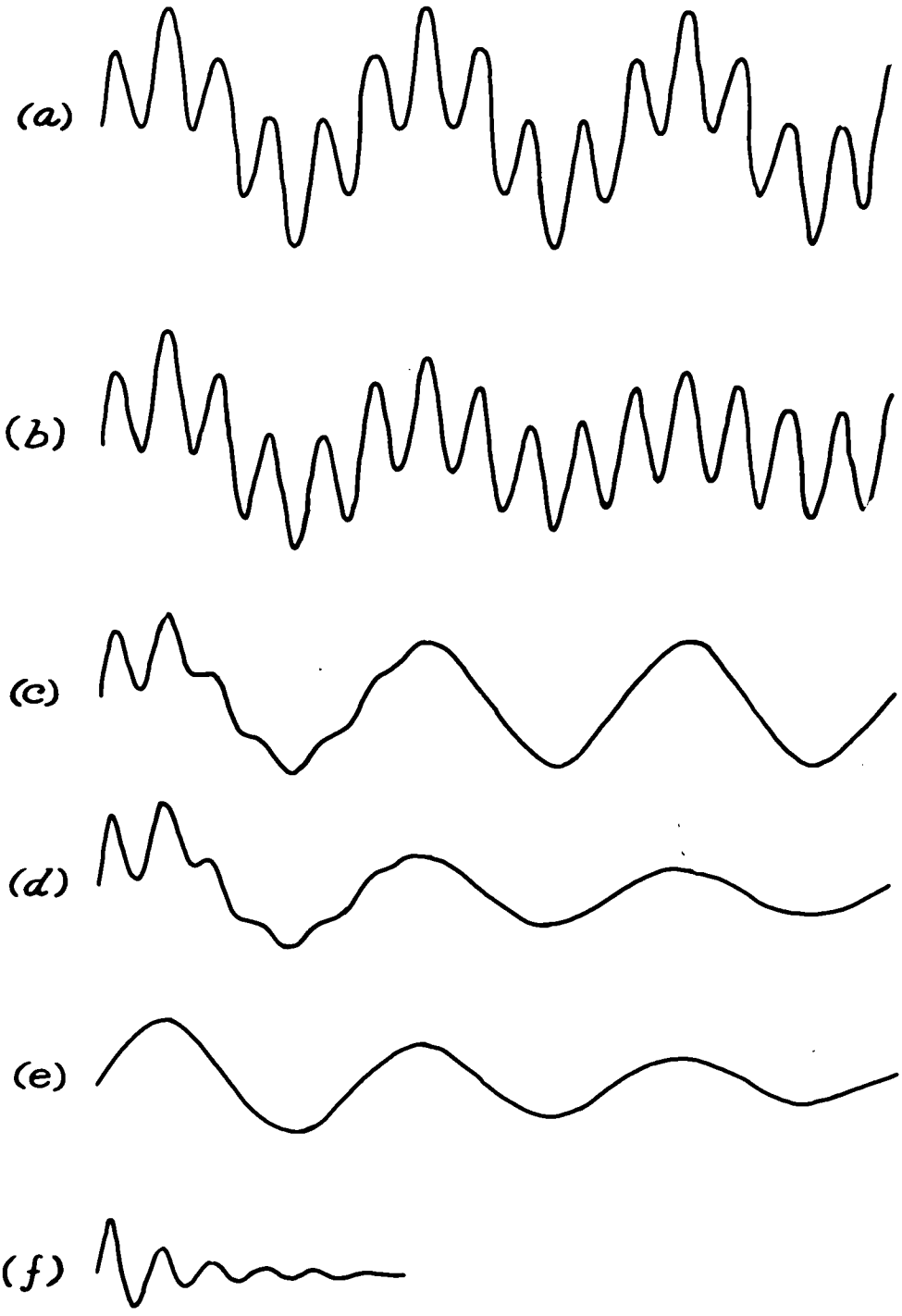
(a) Oscillations of two different periods, both modes undamped.
These could be produced by loops (a) and (b) of Figure 44, one mode of oscillation for each sub-loop.

(b) Oscillations of two different periods, the mode of shorter period being undamped, the other damped.

(c) Oscillations of two different periods, the mode of longer period being undamped, the other damped.
Oscillations (b) and (c) could be produced by loops (a) or (b) of Figure 44, one sub-loop producing sustained (undamped) oscillations, the other only producing damped oscillations.
Oscillations (b) and (c) could also be produced by loop (c) of Figure 44, sub-loop (1) producing the undamped and sub-loop (2) the damped oscillations only.

(d) Both modes of oscillation damped. Characteristic of loops (a), (b) and (c) of Figure 44, both sub-loops producing only damped oscillations.

(e) and (f) Oscillations of only one period.
Characteristic of loops (a), (b) and (c) of Figure 44, the mode of oscillation is one sub-loop being so heavily damped that it is not apparent, only a damped mode of oscillation being produced by the other sub-loop.


(a)
(b)
(c)
(d)
(e)
(f)

it is quite possible that a multiple loop of the type discussed above occurs in bean roots, even though only the sub-loop responsible for the 4 to 7 minute period of oscillation has been investigated so far. The other oscillatory mode of longer periodicity will be studied in Section V.

IV

FURTHER EXPERIMENTAL EVIDENCE OF A BIOELECTRIC FEEDBACK OSCILLATOR

IV

FURTHER EXPERIMENTAL EVIDENCE OF A FEEDBACK OSCILLATION

1. Introduction

In this section it is intended to describe and discuss in turn some additional oscillatory bioelectric phenomena which are consistent with the proposed feedback oscillator discussed in Section III. It is thought that the function of such a feedback loop is to control the physiological variables involved, so maintaining them at certain optimum values. If the physiological condition of the biological system is altered then the dependence of one variable on the next may be changed. This would change the loop parameters such as the gain, so that the amplitude of either spontaneous or resonance oscillations would alter also.

The first effect to be described involves an increase in the total loop gain resulting in an increased tendency for the evocation of bioelectric activity in the form of potential oscillations at the natural period. This occurs after the plant root has been subjected to prolonged excitation at the resonance by the application of an oscillation in osmotic pressure or I.A.A. concentration of the plant's natural period of oscillation. This effect is

similar to some plasticity phenomena exhibited by animal nerve cells (Eccles 1953) in which the threshold of response is lowered and in some cases the response is increased after prolonged stimulation.

2. The Bioelectric Plasticity Effect

In the previous section, the bioelectric potential response to applied oscillations in the osmotic pressure or auxin concentration was described. This response was found to consist in a forced bioelectric oscillation of the same period as the forcing oscillation. It was shown further that the maximum amplitude response or resonance occurred at the plant's natural period of potential oscillation. In addition to this another type of response is possible. In fact it is quite frequently observed that potential oscillations of the natural period are excited by an applied oscillation (either in osmotic pressure or I.A.A.) of considerably different period. This type of response often occurs in plants showing a very marked resonance and the effect is most pronounced at the elongating region of the root. In many plants the effect only appears after the plant has been subjected to excitation at the resonant period for a considerable number of cycles.

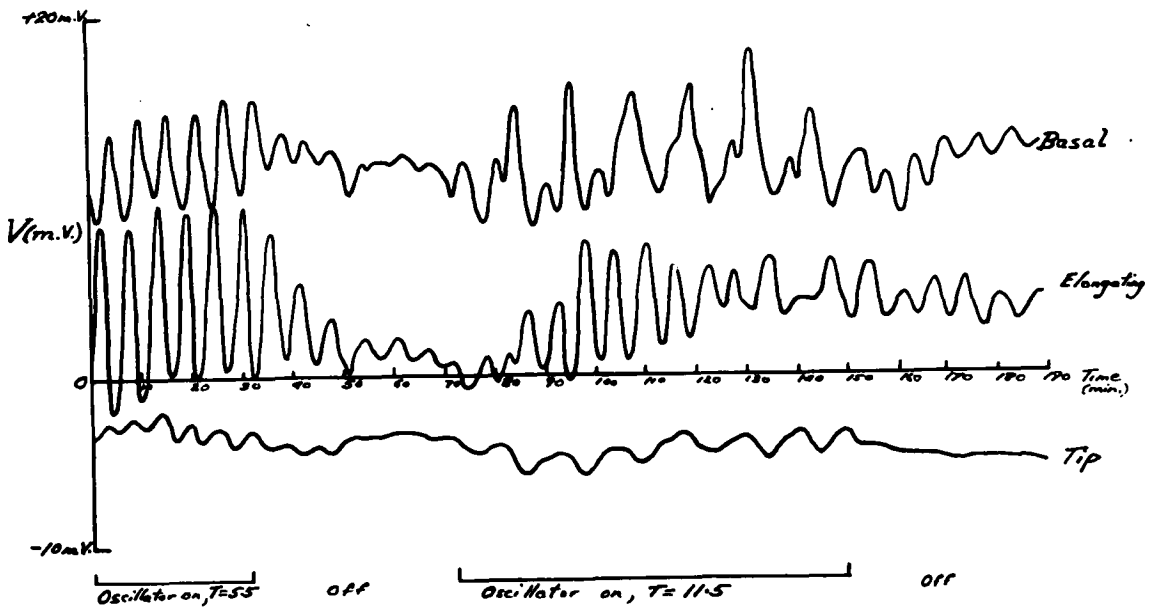
Figure 46 shows the effect quite clearly. Initially resonance was evoked by applying an osmotic pressure oscillation of the natural period ($\simeq$ 5.5 minutes). After this oscillation was removed and

FIGURE 46

Time course of extracellular potentials recorded at the root tip, the elongating zone and the basal region (mature cells) in response to two different periods of osmotic pressure oscillation applied successively.

Initially resonance was evoked for a large number of cycles (including part before commencement of figure) by oscillating the sucrose solution (0 to M/30) at the plant's natural period (5.5 minutes). The osmotic pressure oscillation was then stopped and the potential oscillations at the natural period were damped out.

The osmotic pressure was then oscillated at a period of 11.5 minutes and later was stopped as shown. Different responses at the different root regions are evident.


+20m.V.
V(m.V.)
-10m.V.
Basal
Elongating
Tip
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
Time (min.)
Oscillator on, T=55
off
Oscillator on, T= 11·5
off

the natural oscillation had been damped out, a period of 11.5 minutes was applied. At the tip only the applied period appeared in the potential response whereas at the basal region both the applied and the natural period appeared in the resulting potential variations. At the elongating region the applied period of 11.5 minutes was almost completely absent from the potential response, only oscillations of the natural period being excited. On removal of the 11.5 minute oscillation, the potentials at the elongating and basal regions continued to oscillate at the natural period only, while at the tip no oscillation ensued.

In Section III, 3, it was shown that damped oscillations of the natural period are frequently evoked by a sudden change in osmotic pressure or auxin concentration. Hence it is not surprising that any continuous variation (such as an oscillation) in these two environmental variables might result in bioelectric oscillations of the natural period. However, the increased tendency for oscillations of the natural period to appear after the plant has been subjected to prolonged excitation at resonance is a separate phenomenon. To study this effect in more detail the plant was stimulated to evoke transient oscillations before and after prolonged excitation at the resonance.

It was found that after resonance excitation, the natural

oscillations in the transient were of greater amplitude and were less damped than those evoked by the same stimulus before resonance excitation. The traces on the left and right of Figure 47 show the transient potential oscillations evoked by increasing the osmotic pressure by a constant amount before and after osmotic excitation at the resonance for 3 different plants. Both tracings (left and right) were recorded at the same position along the plant (i.e. the same distance from the root tip). The main feature is the increased amplitude and the decreased damping of the transient oscillations on the right (after resonance) compared with those on the left (before resonance). Results similar to those in Figure 47 were obtained when I.A.A. was used to evoke the transients and the resonant oscillations by suddenly increasing its concentration in the former and oscillating its concentration at the natural period in the latter.

The damping coefficients (logarithmic decrements) of the transient oscillations evoked by various stimuli have been measured both before and after resonance excitation. It has been found that the damping of these transient oscillations does not depend on the manner in which they were stimulated, whether this stimulus be mechanical, electrical, exposure of the root to air or changes in osmotic pressure or I.A.A. concentration. In Figure 48 these damping coefficients, expressed as logarithmic decrements, have been grouped

FIGURE 47

Time courses of extracellular potentials evoked by sudden changes in osmotic pressure (shown by vertical lines). The traces on the left and right show the transient potential oscillations evoked by increasing the osmotic pressure by a constant amount before and after osmotic excitation at resonance for three different plants. Both tracings (left and right) were recorded at the same position along the plant (i.e. the same distance from the root tip).

In the top and middle pairs the osmotic pressure change was from M/60 to M/30 while for the bottom pair, from 0 to M/30.

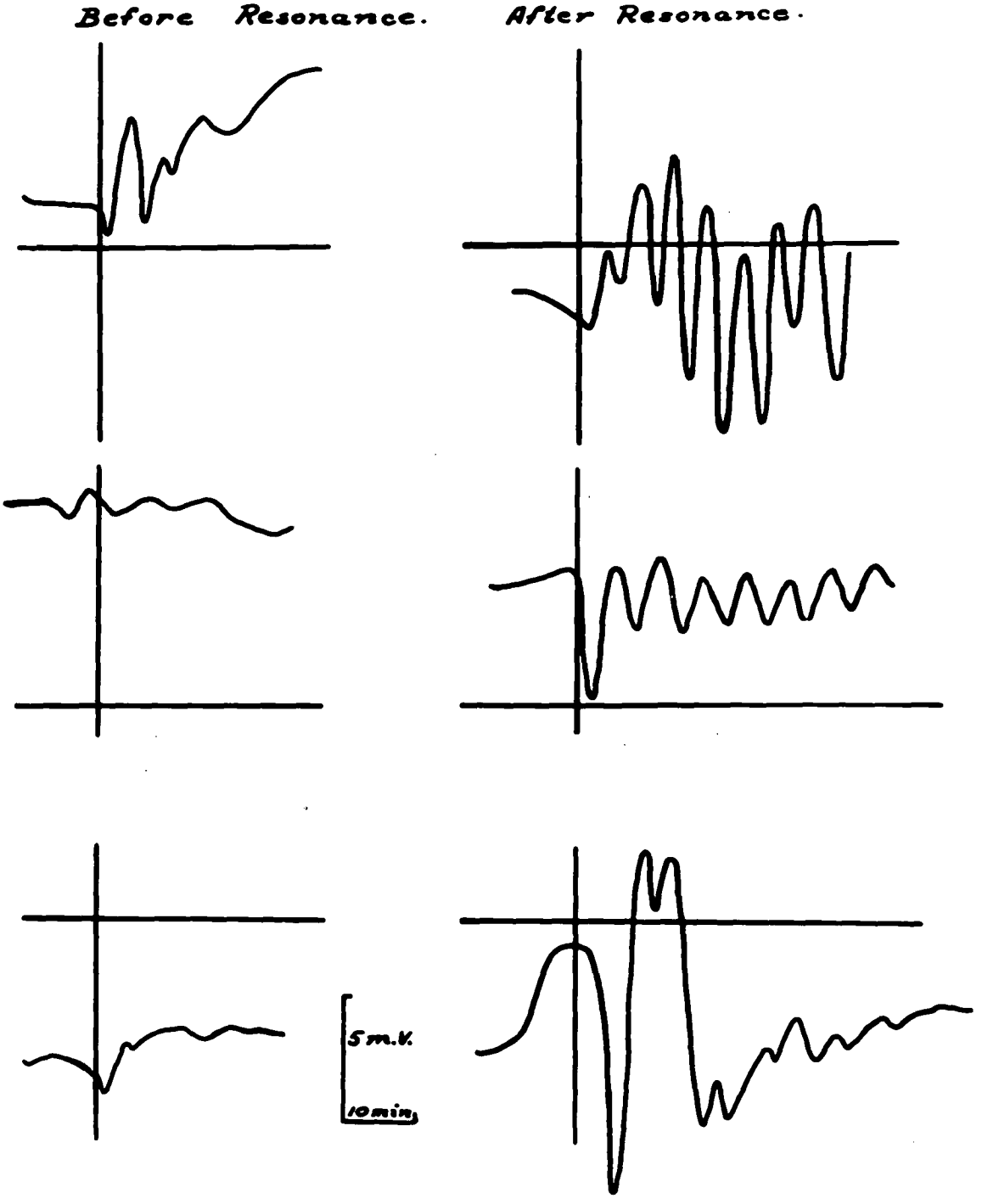
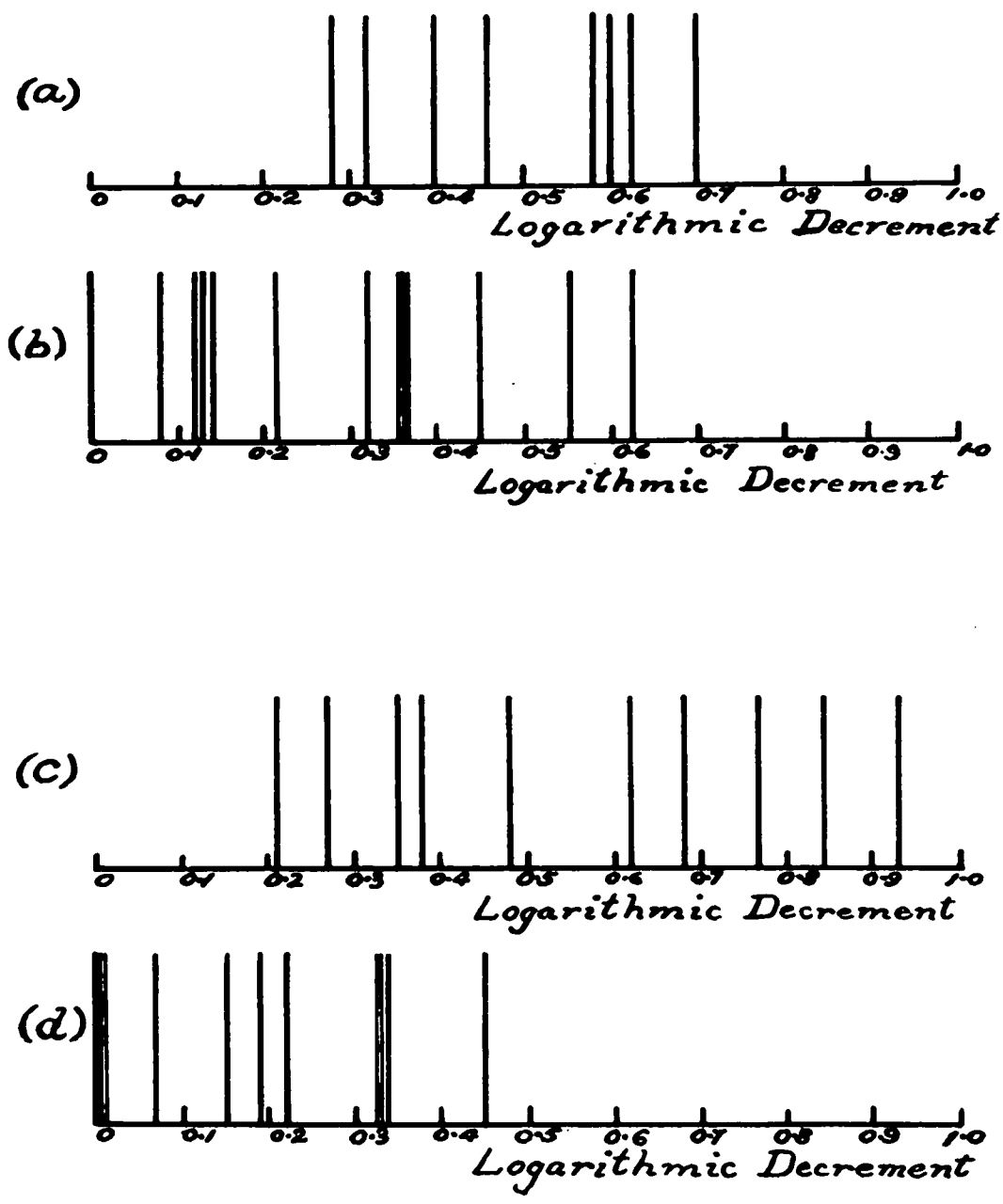

Before Resonance.
After Resonance.
5 m.V.
10 min.

FIGURE 48

The occurrence of logarithmic decrements (shown by vertical lines) of free oscillations, as in Figure 47, for a number of plants. In (a) the transient extra-cellular potential oscillations were evoked before osmotic excitation at the resonance (natural period) while in (b), after resonance.
(c) and (d) show the corresponding results for the logarithmic decrements before and after resonant excitation by I.A.A. solution concentration oscillation (0 to 10^{-7}M).


(a)
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
Logarithmic Decrement
(b)
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
Logarithmic Decrement
(c)
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
Logarithmic Decrement
(d)
0
0·1
0·2
0·3
0·4
0·5
0·6
0·7
0·8
0·9
1·0
Logarithmic Decrement

according as the transient oscillations were evoked before or after resonance excitation by means of an applied oscillation in osmotic pressure or I.A.A. concentration. Before resonance, the logarithmic decrements, as a group, are larger than they are after prolonged excitation at the resonance, whether by means of osmotic pressure or I.A.A. concentration oscillations.

3. Discussion of the Bioelectric Plasticity Effect

In Section III, 8, it was shown that the damping of natural oscillations in the variables of a feedback system is related to the total loop amplification factor K. For a negative feedback system it was shown that as the damping decreases, the magnitude of K increases until at $K = -8$ there is no damping and the system may oscillate spontaneously. Hence the increased amplitude and decreased damping of transient oscillations following resonant excitation may be formally ascribed to an increase in the magnitude of K, the total amplification of the feedback loop. This in turn could be caused by an increase in the magnitude of one or more of the individual amplification factors k_1, k_2 or k_3. A change in the time delays τ_1, τ_2 or τ_3 could also alter the damping.

Such alterations in the amplification factors or the time delays would cause some change in the natural period of oscillation but this would probably be small. No significant changes in period

have been observed in oscillations before and after resonance. Reference to Figure 39(b)(III,8) shows that the change in the natural period (or actually T_H/τ denoted by the position of the vertical arrow on the T/τ axis) for a change in K from -4 to -8, though not negligible, is not large. Further, this change in K, from -4 to -8, means a change from very heavy damping (logarithmic decrement of 0.9) to zero damping. Such a change in damping is considerably more than the damping decreases normally observed after prolonged resonance excitation.

The biological significance of these changes in the formal parameters of the model may be sought in a facilitation of the influence of one physiological variable on another, e.g. the effect of auxin on membrane permeability to ions. By subjecting the membranes to large oscillatory variations in auxin concentration, as envisaged at resonance, the membrane structure may well be modified so as to render its permeability more sensitive to changes in auxin concentration. The facilitation of other such interactions between variables suggested in the model is also conceivable.

4. The Excitatory and Inhibitory effects of Auxins and Antiauxins on Bioelectric Oscillations

Since the distribution of auxin in the tissue is considered to be one of the variables in the feedback loop, it might well be

expected that if the normal mode of auxin transport and biochemical action were altered, then the mode of operation of the feedback loop oscillator would also change. It is well known that auxin itself, in strong concentrations, is self-inhibitory in that the physiological action of auxin in optimum concentrations is destroyed by increasing its concentration too much (McRae and Bonner 1953). Auxin antagonists such as 26DCPA* are known to inhibit the normal action of auxin by competing for the site of chemical action of the auxin molecule (McRae and Bonner 1953). Other substances such as T.I.B.A.† are found to inhibit the transport of natural auxin in vivo (Hay 1931).

In this section it is proposed to describe and discuss the effects of these substances, known generally as "antiauxins", on the bioelectric potentials of plant roots. Although some experiments were undertaken using the auxin transport inhibitor T.I.B.A., the major part of this section is devoted to a comparison of the effects evoked by auxins and auxin antagonists when added to the bathing solution of the plant in both constant and oscillatory concentrations.

The results of these experiments are discussed in terms of the known physiology of auxins and antagonists. It is shown that these results together with their physiological interpretation have considerable bearing on the relation of auxin to the proposed feedback loop responsible for bioelectric oscillations. The

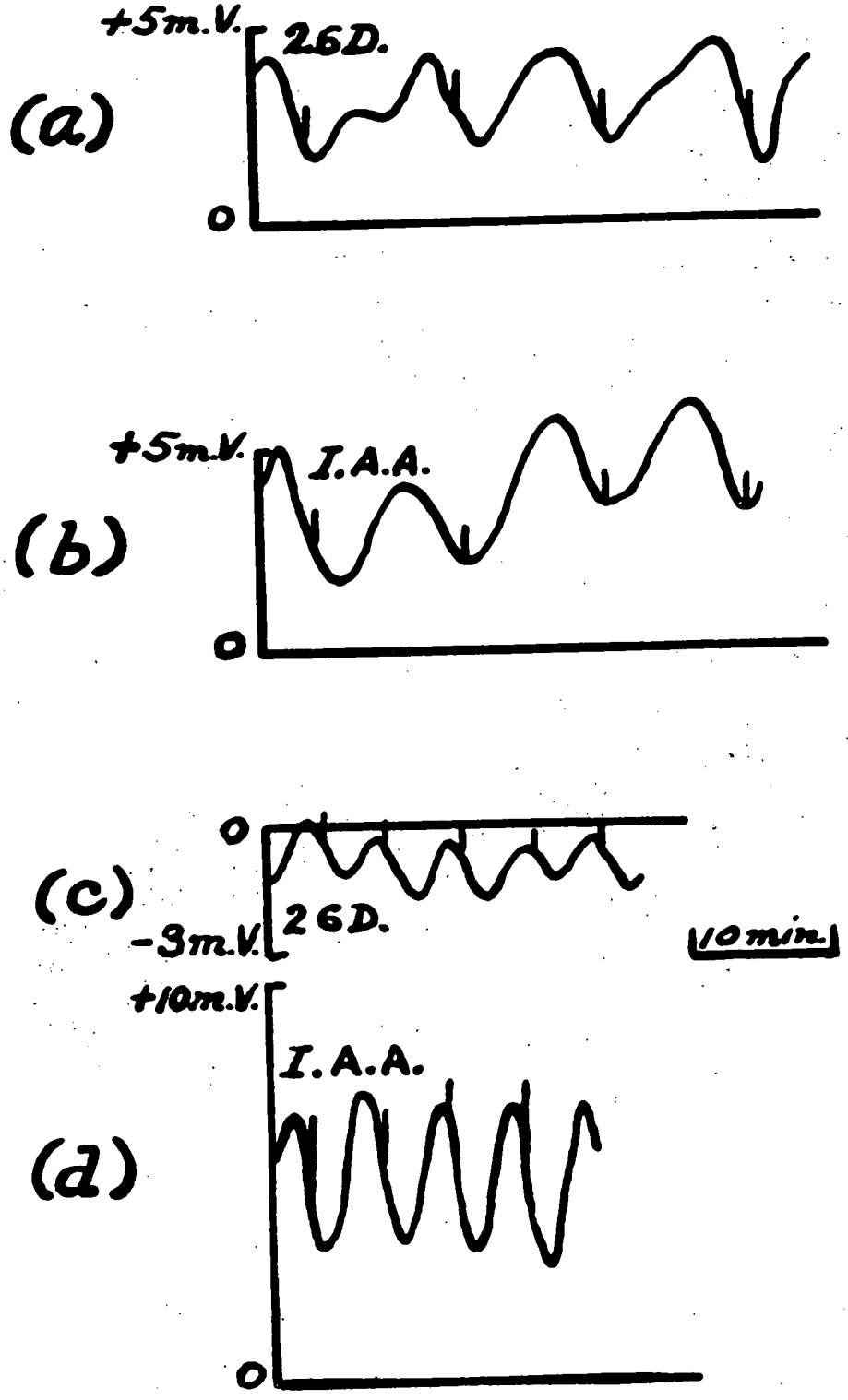
*2,6 - Dichlorophenoxyacetic acid
†2,3,5 - Triiodobenzoic acid

oscillatory potential responses evoked by oscillating the concentration of auxin (I.A.A.) in the bathing solution of a plant root have already been discussed.

The oscillations shown in Figure 49(a) were produced in response to an applied oscillation in the concentration of an auxin antagonist 26D.C.P.A. in the plant root's bathing solution. The times of maximum 26D.C.P.A. concentration (viz. 10^{-7}M) are indicated by the vertical marks on the record. Immediately after this record was obtained the 26D.C.P.A. was replaced by I.A.A. and some minutes later the record of the potential response from the same plant (Figure 49(b)) was obtained. In both cases the maximum concentration of 26D.C.P.A. and of I.A.A. in the cycle were 10^{-7}M. The potential responses in the two cases are very similar as regards amplitude and phase with respect to the applied oscillation. Figures 49(c) and (d) show potential oscillations evoked by 26D.C.P.A. and I.A.A. concentration oscillations at another period. Again the responses were recorded from the same plant but in this case the I.A.A. response was obtained about four hours after the 26D.C.P.A. response. Although the average value of the potential changed sign during this time and the potential oscillations differ in amplitude, it is evident that the same phase relations exist with respect to the applied concentration oscillations in both cases.

FIGURE 49

Time courses of the extracellular potential response to oscillations in the concentration of the auxin antagonist 2δ D.C.P.A. (a) and the auxin I.A.A. (b), recorded on the same plant and at the same period of oscillation. Similar results are shown in (c) and (d) for a different period of oscillation.
In all cases the concentration of the auxin or antagonist was oscillated between 0 and 10^{-7}M (shown by the vertical strokes on the traces).


+5m.V.
26D.
(a)
0
+5m.V.
I.A.A.
(b)
0
0
(c)
-3m.V.
26D.
10 min.
+10m.V.
I.A.A.
(d)
0

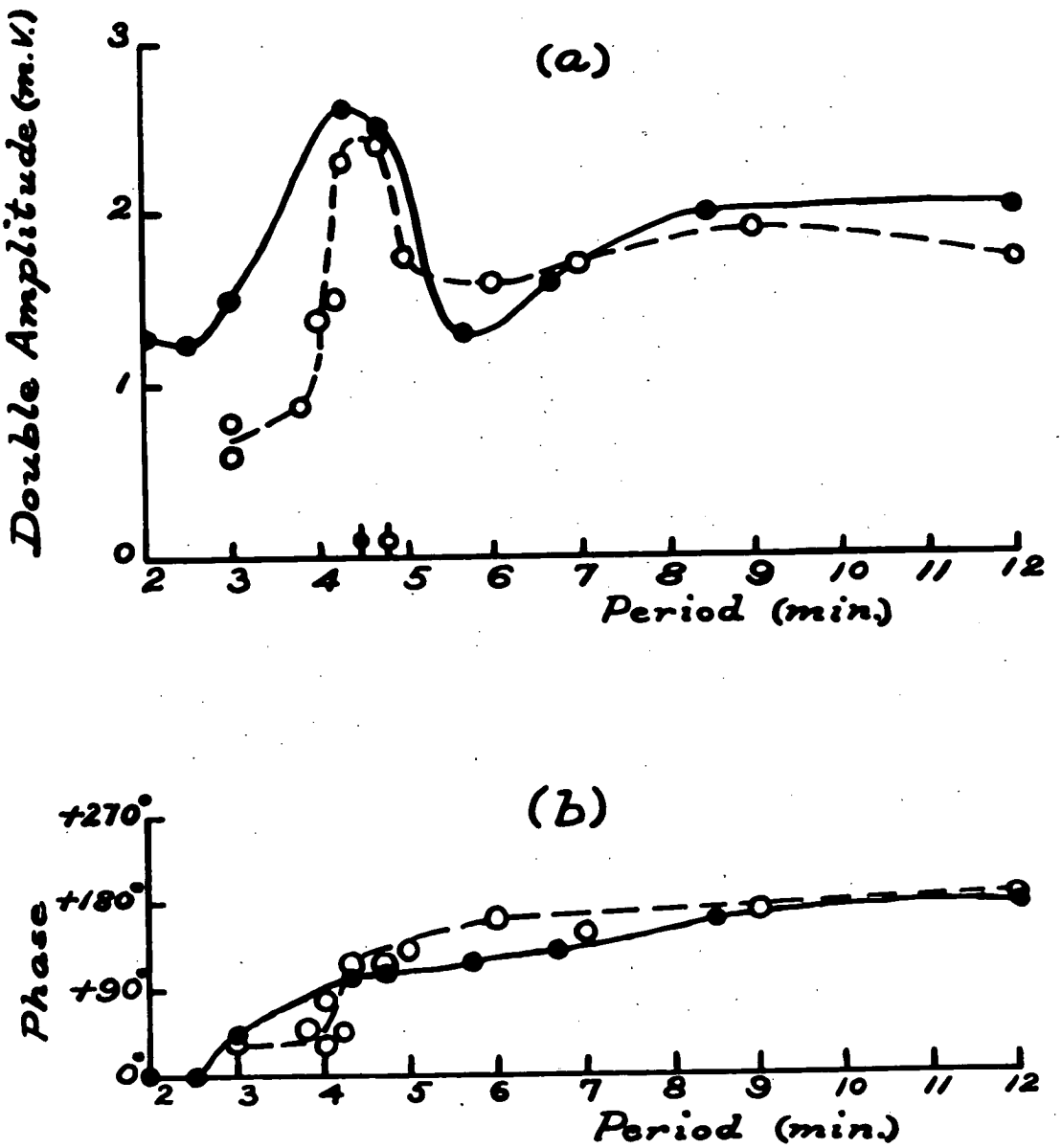
Figures 50(a) and (b) show the amplitude and phase ~~and~~ response for typical plants to oscillations of varying period in the applied concentration of the auxin antagonist 2&D.C.P.A. The peak concentration for all cycles and periods was 10^{-7}M. The main features again are the same as for I.A.A.: resonance occurs at the natural period of oscillation and the phase change from short to long periods is 180°.

From these results it appears that for the concentrations involved an applied oscillation in auxin or auxin antagonist evokes substantially the same bioelectric response.

It is found that the addition of relatively weak concentrations (below 10^{-6}M) of the auxin I.A.A. to the root's bathing solution does not affect spontaneous potential oscillations. It was seen however to stimulate oscillations in cases where the oscillations were small or non-existent. At 10^{-6}M however the amplitude of the oscillations is decreased while the addition of 10^{-5}M I.A.A. inhibits the oscillations completely. This is shown in Figure 51(a) in which concentrations of 10^{-9}M, 10^{-7}M, 10^{-6}M, 3×10^{-6}M and 10^{-5}M I.A.A. were added successively to the bathing solution of a root producing spontaneous potential oscillations following resonance. These results are paralleled by the fact that routine observations of growth rate have revealed no changes in the normal extension of

FIGURE 50

The relations between the double amplitude (a) and the phase (b) responses of the extracellular potentials at the elongating regions and the period of oscillation of 26 D.C.P.A. concentration (0 to 10^{-7} M) for two plants. The natural periods of oscillation for the two plants are shown on the period axis of (a).


(a)
Double Amplitude (m.V.)
3
2
1
0
2
3
4
5
6
7
8
9
10
11
12
Period (min.)
(b)
Phase
+270°
+180°
+90°
0°
2
3
4
5
6
7
8
9
10
11
12
Period (min.)

the plant root in concentrations of I.A.A. less than 10^{-6}M. The elongation of the root ceases however on addition of 10^{-5}M I.A.A.

The effect of adding I.A.A. to the bathing solution of a plant root producing potential oscillations in response to an osmotic pressure oscillation is the same as that for spontaneous potential oscillations. The potential record shown in Figure 51(b) was obtained by applying an osmotic pressure oscillation (by varying the concentration of Mannitol in the bathing solution between M/30 and zero) to the plant root and successively adding increased concentrations of I.A.A. to the bathing solution. The osmotic pressure oscillation, at the resonant period, was continued throughout. The addition of 10^{-9}M and 10^{-7}M I.A.A. caused no appreciable change in the amplitude of the potential oscillations but at 10^{-5}M I.A.A. the oscillations were damped and disappeared after a few more cycles.

In Figure 51(c) the results of applying oscillations of the resonant period in the concentration of I.A.A. to the root's bathing solution are shown. Initially the oscillation in I.A.A. concentration was between zero and 10^{-7}M. This was then changed to an oscillation between zero and 10^{-6}M I.A.A. This produced no appreciable change in the amplitude of the potential oscillations. The I.A.A. concentration was then oscillated between zero and 10^{-5}M. The potential oscillations disappeared.

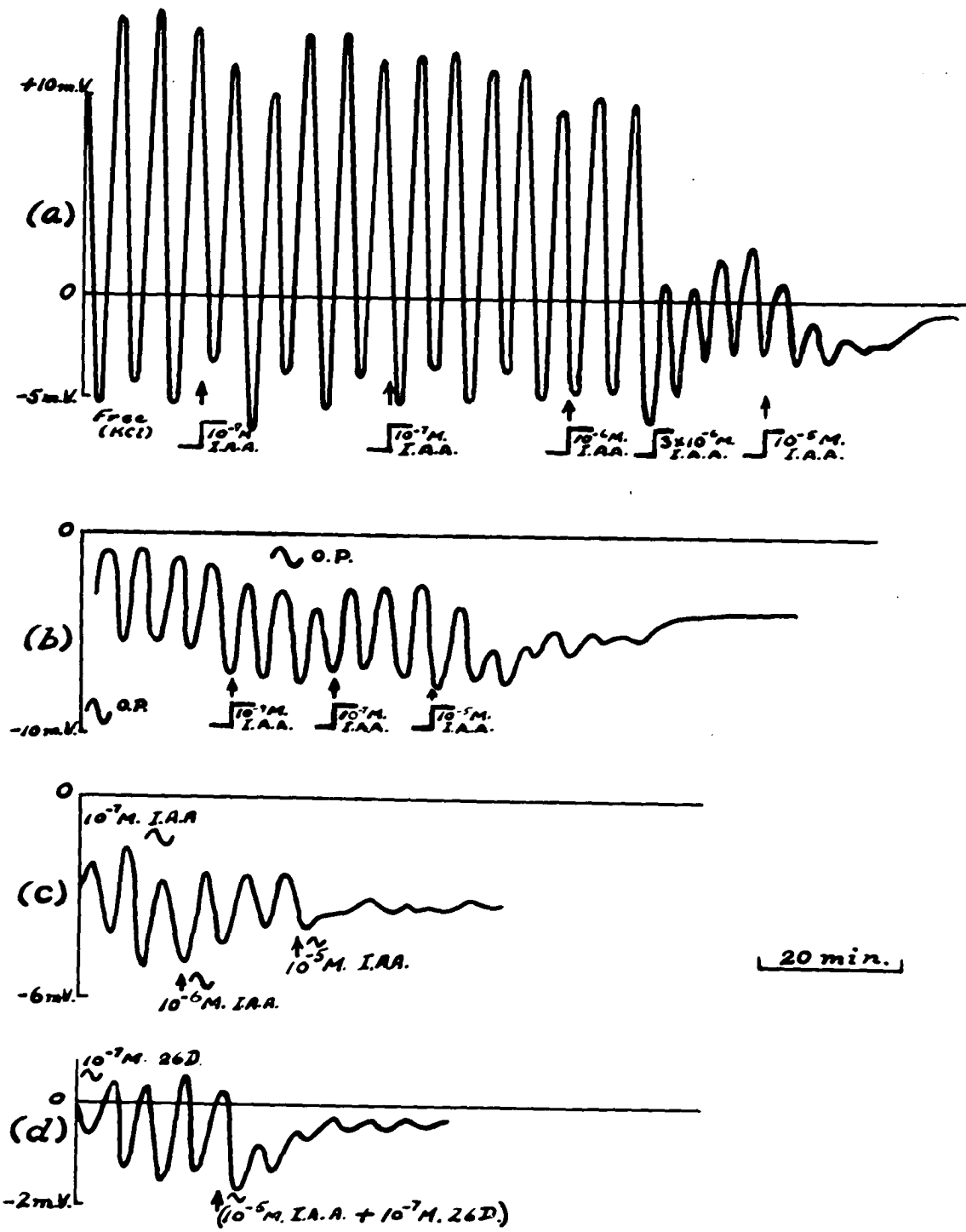
FIGURE 51

(a) The effect of increasing the bathing solution concentration of I.A.A. in successive steps. Initially the plant root was producing spontaneous extracellular potential oscillations in 10^{-4}M KCl following resonance.
The I.A.A. concentration was increased suddenly to the values shown at the times marked by arrows and ⌐ .

(b) The effect of increasing the bathing solution concentration of I.A.A. in successive steps to a plant producing resonant potential oscillations (extracellular) evoked by an oscillation in osmotic pressure applied throughout, represented symbolically by ∿ O.P.

(c) The effect of successively increasing the peak value of the oscillatory concentration of I.A.A. applied to the bathing solution of the root. The applied period of oscillation was the natural (resonant) period for the plant root.

(d) The effect of applying an oscillation in I.A.A. solution concentration (0 to 10^{-5}M) in addition to and in phase with the oscillation in 26 D.C.P.A. (0 to 10^{-7}M) applied throughout. Both solution concentration oscillations were applied at the plant's natural period.


+10m.V.
(a)
0
-5m.V.
Free (KCl)
10⁻⁹M. I.A.A.
10⁻⁷M. I.A.A.
10⁻⁶M. I.A.A.
3x10⁻⁶M. I.A.A.
10⁻⁵M. I.A.A.
0
(b)
O.P.
O.R
-10m.V.
10⁻⁹M. I.A.A.
10⁻⁷M. I.A.A.
10⁻⁵M. I.A.A.
0
10⁻⁷M. I.A.A
(c)
10⁻⁵M. I.A.A.
10⁻⁶M. I.A.A.
-6mV.
20 min.
10⁻⁷M. 2,6D.
(d)
0
-2mV.
(10⁻⁶M. I.A.A. + 10⁻⁷M. 2,6D.)

In Figure 51(d) the initial potential oscillation was evoked by applying an oscillation in 26D.C.P.A. concentration between zero and 10^{-7}M in the bathing solution. I.A.A. at 10^{-5}M was then added to the 10^{-7}M 26D.C.P.A. so that the auxin plus auxin antagonist concentration of the bathing solution oscillated between zero and 10^{-5}M I.A.A. plus 10^{-7}M 26D.C.P.A. This inhibited the potential oscillations.

From these results it is apparent that the addition of I.A.A. at concentrations greater than about 10^{-6}M, either in constant or oscillatory concentrations, inhibits the potential oscillations irrespective of the manner in which they were evoked.

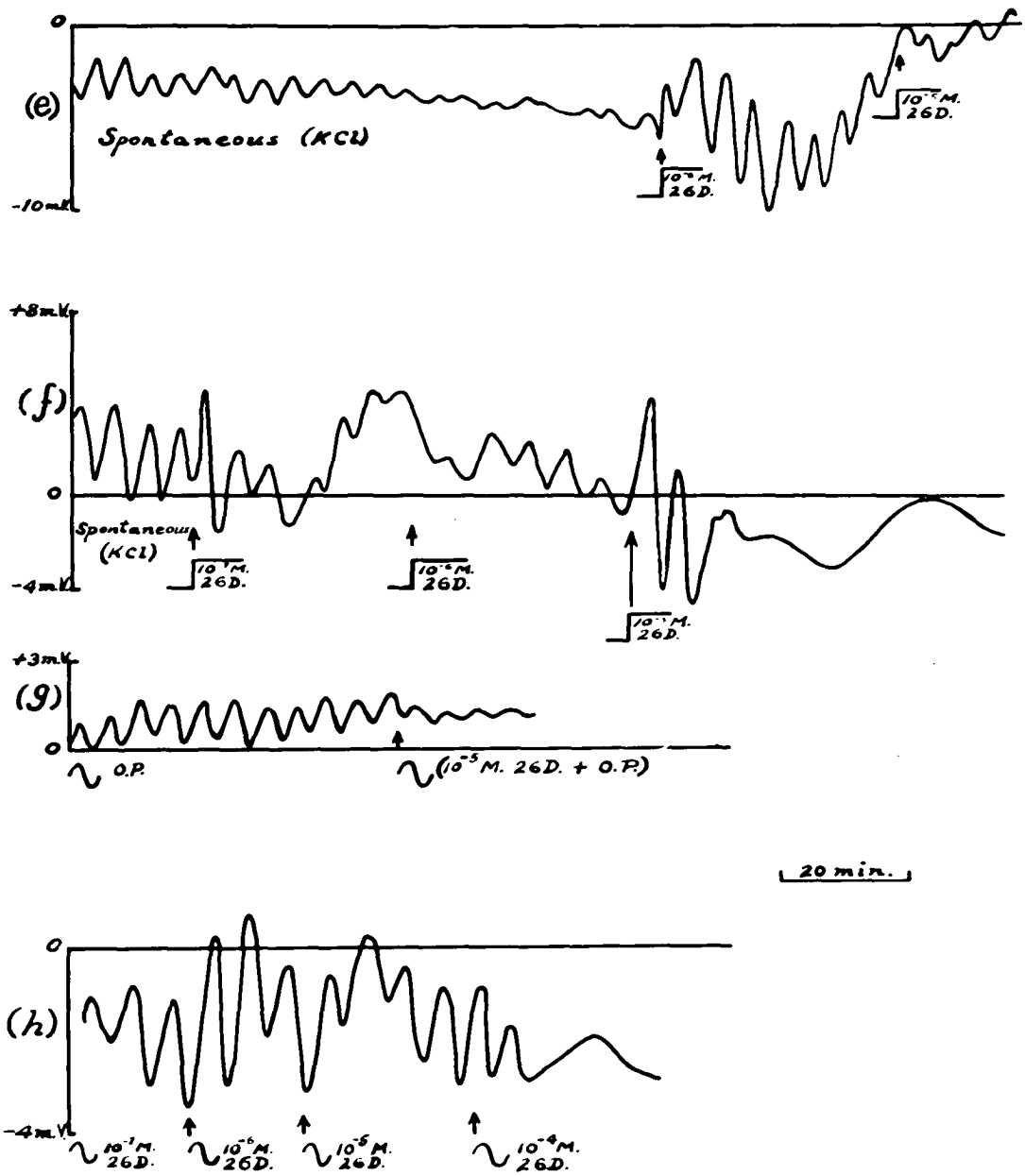
The action of the auxin antagonist 26D.C.P.A. is very similar to that of I.A.A. For a plant capable of producing oscillations, the addition of fairly weak concentrations of 26D.C.P.A. (up to 10^{-6}M) will initiate sustained oscillations in the same manner as for changes in osmotic pressure or auxin (I.A.A.) concentration (provided the I.A.A. concentration is less than about 10^{-6}M) (Section IV, 2). This is shown in Figure 51(e) in which an initial train of spontaneous oscillations were damped out but returned when 10^{-6}M 26D.C.P.A. was added. These oscillations were however inhibited by the addition of 10^{-5}M 26D.C.P.A. Similar results are shown in Figure 51(f) in which the successive addition

FIGURE 51

(e) and (f) The effect of increasing the bathing solution concentration of 26 D.C.P.A. in steps to a plant initially producing spontaneous extracellular potential oscillations in 10^{-4} M KCl.

(g) The effect of applying an oscillation in 26 D.C.P.A. solution concentration (0 to 10^{-5} M) in addition to and in phase with the oscillation in osmotic pressure (0 to M/30) applied throughout.
Both solution concentration oscillations were applied at the plant's natural period.

(h) The effect of successively increasing the peak value of the oscillatory concentration of 26 D.C.P.A. applied to the bathing solution of the root.
The applied period of oscillation was the natural (resonant) period for the plant root.


0
(e)
Spontaneous (KCl)
-10mV
10⁻⁴M. 26D.
10⁻⁴M. 26D.
10⁻⁴M. 26D.
+8mV
(f)
0
Spontaneous (KCl)
-4mV
10⁻¹M. 26D.
10⁻⁴M. 26D.
10⁻⁴M. 26D.
+3mV
(g)
0
O.P.
(10⁻⁵M. 26D. + O.P.)
20 min.
0
(h)
-4mV
10⁻¹M. 26D.
10⁻⁶M. 26D.
10⁻⁵M. 26D.
10⁻⁴M. 26D.

of 10^{-7}M and 10^{-6}M 26D.C.P.A. failed to suppress the spontaneous potential oscillations. Although the addition of 10^{-5}M 26D.C.P.A. caused an increase in the amplitude of the oscillations for a couple of cycles, thereafter the short period oscillations ceased completely.

In Figure 51(g) the potential oscillations were evoked throughout the duration of the record by an osmotic pressure oscillation at the resonant period. The addition of 10^{-5}M 26D.C.P.A. inhibited the oscillations although they still persisted with decreased amplitude.

In Figure 51(h) the potential oscillations were evoked by oscillating the 26D.C.P.A. concentration between zero and the following peak values 10^{-7}M, 10^{-6}M, 10^{-5}M and 10^{-4}M. The 26D.C.P.A. concentration was oscillated at the resonant period throughout. At 10^{-4}M 26D.C.P.A. the oscillations ceased.

In Figure 51(i) the potential oscillations were evoked initially by oscillating the I.A.A. concentration between zero and 10^{-7}M at the resonant period. Then the following concentrations of 26D.C.P.A. were successively mixed with the 10^{-7}M I.A.A.: 10^{-7}M, 10^{-6}M and 10^{-5}M. This means that the concentration of auxin plus auxin antagonist was oscillated successively between zero and 10^{-7}M I.A.A. 10^{-7}M I.A.A. plus 10^{-7}M 26D.C.P.A., 10^{-7}M I.A.A. plus 10^{-6}M 26D.C.P.A., 10^{-7}M I.A.A. plus 10^{-5}M 26D.C.P.A. The resonant

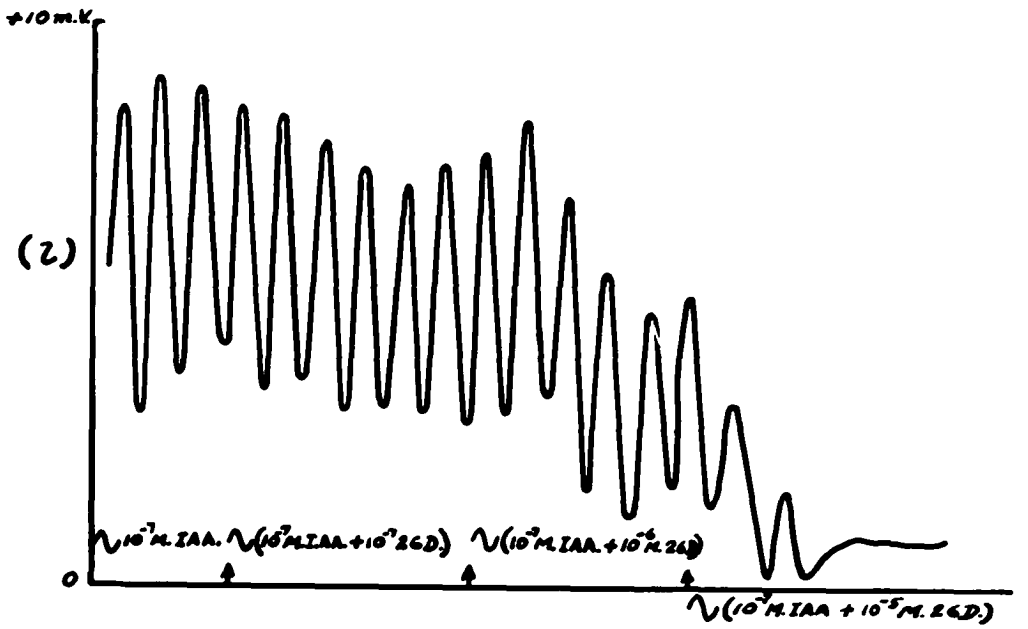
FIGURE 51

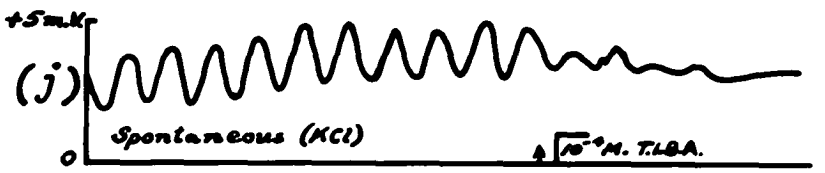
(i) The effect of successively increasing the peak value of the oscillatory concentration of 26 D.C.P.A. in addition to and in phase with the oscillation in I.A.A. solution concentration (0 to 10^{-7}M) applied throughout. Both solution concentration oscillations were applied at the plant's natural period.

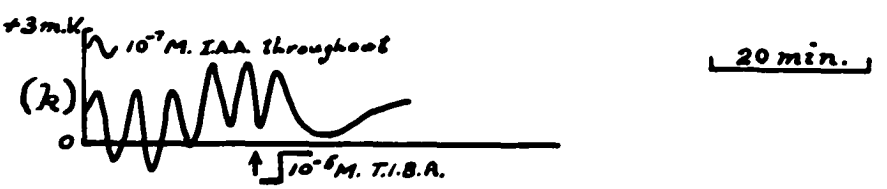
(j) The effect of adding 10^{-4}M T.I.B.A. to the bathing solution (10^{-4}M KCl) of a plant producing spontaneous extracellular potential oscillations.

(k) The effect of adding 10^{-5}M T.I.B.A. to the bathing solution of a plant producing resonant potential oscillations in response to an oscillation in I.A.A. (0 to 10^{-7}M) applied throughout.

In all cases (a) to (k) the constant background bathing solution was 10^{-4}M KCl and the potentials measured were the extracellular potentials at the elongating zone of the root.


+10 m.K
(l)
0
10^{-7} M. IAA.
10^{-7} M IAA + 10^{-7} 2,4 D.
10^{-9} M. IAA + 10^{-6} M. 2,4 D.
10^{-7} M. IAA + 10^{-5} M. 2,4 D.


+5 m.K
(j)
0
Spontaneous (KCl)
10^{-4} M. T.I.B.A.


+3 m.K
10^{-7} M. IAA throughout
(k)
0
10^{-6} M. T.I.B.A.
20 min.

potential oscillations were not inhibited until the 26D.C.P.A. oscillatory concentration reached 10^{-5}M at which the potential oscillations disappeared rapidly.

These results show that although the application of oscillatory concentrations of 26D.C.P.A. less than about 10^{-5}M peak value evoke potential oscillations, the application of 26D.C.P.A. concentrations 10^{-5}M and greater, either in constant or oscillatory form, inhibit the potential oscillations independent of the manner of their initial evokation. Thus the inhibitory action of the auxin antagonist is the same as that of the auxin I.A.A.

It was found that the addition of T.I.B.A. inhibited both spontaneous oscillations (Figure 51(j)) and potential oscillations evoked by oscillating the I.A.A. concentration between zero and 10^{-7}M at the resonant period (Figure 51(k)). In Figures 51(j) and (k) the constant concentrations of T.I.B.A. were 10^{-4}M and 10^{-5}M respectively. These results show that the auxin transport inhibitor also inhibits potential oscillations, whether spontaneous or forced.

5. Discussion of the Excitatory and Inhibitory Effects of Auxin and Auxin Antagonists on Bioelectric Oscillations

Before discussing the bioelectric affects evoked by auxins and auxin antagonists in the root's bathing solution either in

constant or oscillatory concentrations, it is necessary to consider briefly some of the known biochemical and physiological facts concerning these substances. These have been summarised by McRae and Bonner (1953).

The biochemical action of auxin in producing cell wall plasticity is thought to consist initially in the attachment of an enzyme to the auxin molecule, the attachment taking place at two points on each molecule. Cell enlargement then results from the osmotic uptake of water, so stretching the plasticised cell wall.

It is possible however for an auxin molecule to become attached to its enzyme at only one point, in which case cell wall plasticisation does not ensue. The molecules of auxin antagonists are similar to auxin molecules but one of the sites of attachment to the enzyme is either absent or inactivated. Consequently the auxin antagonist molecule can form only a one-point attachment to an auxin enzyme. Thus if auxin molecules are present in the tissue, either naturally or artificially supplied, the added antagonist competes for the auxin enzymes, so inhibiting the true auxin action.

Auxin in sufficiently strong concentrations is self-antagonistic in that the auxin molecules compete for the enzymes, one site of one auxin molecule becoming attached to one point on the enzyme molecule and one site of another auxin molecule becoming attached to the other point on the same enzyme molecule.

The exact physiological action of auxin in relation to bioelectric potentials is of course not known. However the relation of auxin to bioelectric potential oscillations and that of auxin to cell wall plasticity and consequent cell enlargement occur in the same morphological region of the root. Further, in the experiments described, there is a close parallel between the actions of artificially supplied auxins and antagonists on both the bioelectric potential behaviour and on root extension. Hence it seems probable that a similar biochemical action of auxin is involved in both cases. It may well be that the same initial chemical action between auxin and its enzyme leads eventually to both cell enlargement and the affects of auxin on the oscillatory bioelectric potentials.

There seems to be no reliable evidence to suggest that increased elongation of intact roots is caused by the addition of auxins in concentrations of 10^{-9}M or greater to the root's bathing solution. In fact, Åberg (1957) has strongly criticised the various claims, that increased elongation of intact roots is stimulated by the addition of auxins in any concentration between about 10^{-18}M and 10^{-3}M. Burstrom (1950, 1951[a], 1951[b]) has shown that various auxin antagonists used in concentrations ranging from 10^{-8}M to 10^{-5}M inhibit the elongation of wheat roots for several hours, thereafter enhanced elongation occurs. No significant evidence for increased

elongation rates of roots subjected to I.A.A. between 10^{-9}M and 10^{-5}M or to 26D.C.P.A. between 10^{-9}M and 10^{-4}M has been obtained in routine observations in the experiments described in this section. In fact root elongation ceased when the concentration of I.A.A. or 26D.C.P.A. was increased to about 10^{-5}M.

Measurements of the concentration of auxin inside intact roots suggest that this is higher than the concentrations of auxin which produce increased elongation in root segments where the auxin concentration can be controlled. (Åberg, 1957). It seems probable therefore that the addition of I.A.A. in concentrations even as low as 10^{-9}M is partially inhibitory to root extension. That is, the total concentration of auxin molecules is sufficiently high to cause partial inhibition of the auxin two-point attachment to its enzyme. In other words the addition of auxin acts in the same way as the addition of auxin antagonists. Hence an increase in concentration of either auxin or auxin antagonist decreases the number of two point attachments taking place between auxin molecules and their enzymes in the root tissue. Conversely a decrease in concentration of either increases the number of two-point attachments taking place. Consequently it is quite reasonable that the action of both auxin and auxin antagonists should be very similar so far as their effects on the bioelectric potentials of roots are concerned.

This would explain the fact that the application of oscillatory concentrations of I.A.A. and 26D.C.P.A. (provided the peak concentrations are sufficiently weak so as to cause no appreciable inhibition of root elongation) evokes oscillations in the plant root potentials, the phases of the potential oscillations being the same for I.A.A. and 26D.C.P.A. for a given period of the applied oscillation.

In Section IV, 4, it was seen that if the concentrations of I.A.A. or 26D.C.P.A. are weak(i.e. sufficiently low that no appreciable inhibition of root elongation occurs), changes (either increasing or decreasing) in the concentration of either auxin or antagonist will initiate damped, and occasionally sustained, potential oscillations. If sustained potential oscillations either spontaneous or resonant are already present, changes in the concentration of I.A.A. or 26D.C.P.A. have no effect. These results, again the same for auxin and antagonist, may be explained in terms of the change in the number of two-point attachments taking place between auxin molecules and their enzymes caused by a change in the amount of either auxin or antagonist applied. That is, a change in either causes a change in the effective auxin concentration in the tissue.

If the auxin supply and distribution be considered as one of the variables in a negative feedback loop, a change in effective auxin concentration would lead to changes in the other loop variables such that the auxin supply would be adjusted to oppose the initial

change. If the loop is at all unstable this automatic adjustment would be of a damped oscillatory form. Hence oscillations, either damped or possibly sustained would appear in all the loop variables such as those observed in the bioelectric potential.

If the variables of the loop, including the bioelectric potential, are already in oscillation, either spontaneous (because of loop instability) or in forced resonance, the amplitude of the oscillations is controlled by the feedback loop parameters such as the total gain and possibly by nonlinear characteristics. Consequently a change in auxin concentration (or an effective change by addition of an auxin antagonist) would not be likely to change the amplitude of the oscillations, unless the loop parameters were also changed. This again is unlikely if the auxin and auxin antagonist concentrations are weak enough not to affect the elongation of the root appreciably.

If the concentration of either auxin or antagonist is increased so much that two-point attachments between auxin molecules and their enzymes are totally inhibited, then the feedback loop is effectively broken at the point where auxin action is involved. For the auxin supply then becomes an invariant so that the feedback action of the loop is prevented. The prevention of auxin transport by means of T.I.B.A. so prohibiting variations in the auxin supply would have the same effect of inactivating the feedback oscillator. Consequently no oscillations in the bioelectric potential would be produced.

V

TRANSIENT ANALYSIS OF LONG PERIOD POTENTIAL OSCILLATIONS

V

TRANSIENT ANALYSIS OF LONG PERIOD POTENTIAL OSCILLATIONS

1. Introduction

In Section III, 8, it was shown that the differential equation describing the variables of a feedback loop with three delays is of the third order. The solution of this equation contains an exponential term and an oscillatory term which may be damped. For a two delay system a second order equation yielding only a damped oscillatory solution with no exponential term results. An examination of the typical transient potentials in Figure 9(b) shows that each contains a damped oscillation together with an exponential component. This suggests that transients containing the long period oscillations can be described by a third order differential equation. It is probable therefore that these long period oscillations also result from a three delay feedback loop which is however distinct from that responsible for the shorter period (4 to 7 minutes) oscillations. The two loops may however be coupled to form a double loop such as those in Figures 44(a) and (b) (Section III,12).

In principle it would be possible to investigate the proposed long period feedback loop by the frequency response method used in

Section III for the shorter period oscillations. However in practice it would be impossible to examine the same morphological regions of the plant throughout, because of its growth. Consequently the method of transient analysis has been employed to study the long period oscillations.

The method depends on the fact that by making only a limited number of assumptions about a proposed type of feedback loop, the period of oscillation can be calculated from the measured values of the time constant of the exponential component and the damping of the oscillations in the transient. By comparing the calculated value of the period with the actual period of the oscillations in the transient, the validity of the assumptions regarding the feedback loop may be tested.

2. Theory of Transient Analysis

It is assumed as a first approximation that the negative feedback loop has three equal time delays. No assumptions regarding the amplification factors k_1, k_2, k_3 or their product K are necessary. In Section III,8, equation (17) it was shown that the transient response of the variables of such a system is given by

$$p = p_o e^{+\alpha t/\tau} + p_1 e^{+\beta t/\tau} \cos\left(\frac{2\pi t}{T_n}\right) \qquad (17)$$

where

$$T_n = 2\pi\tau / \sqrt{\gamma^2 - \beta^2} \tag{18}$$

and

$$-\alpha\gamma^2 = 1 - k_1k_2k_3 = 1 - K \tag{19}$$

$$-\alpha - 2\beta = 3 \tag{20}$$

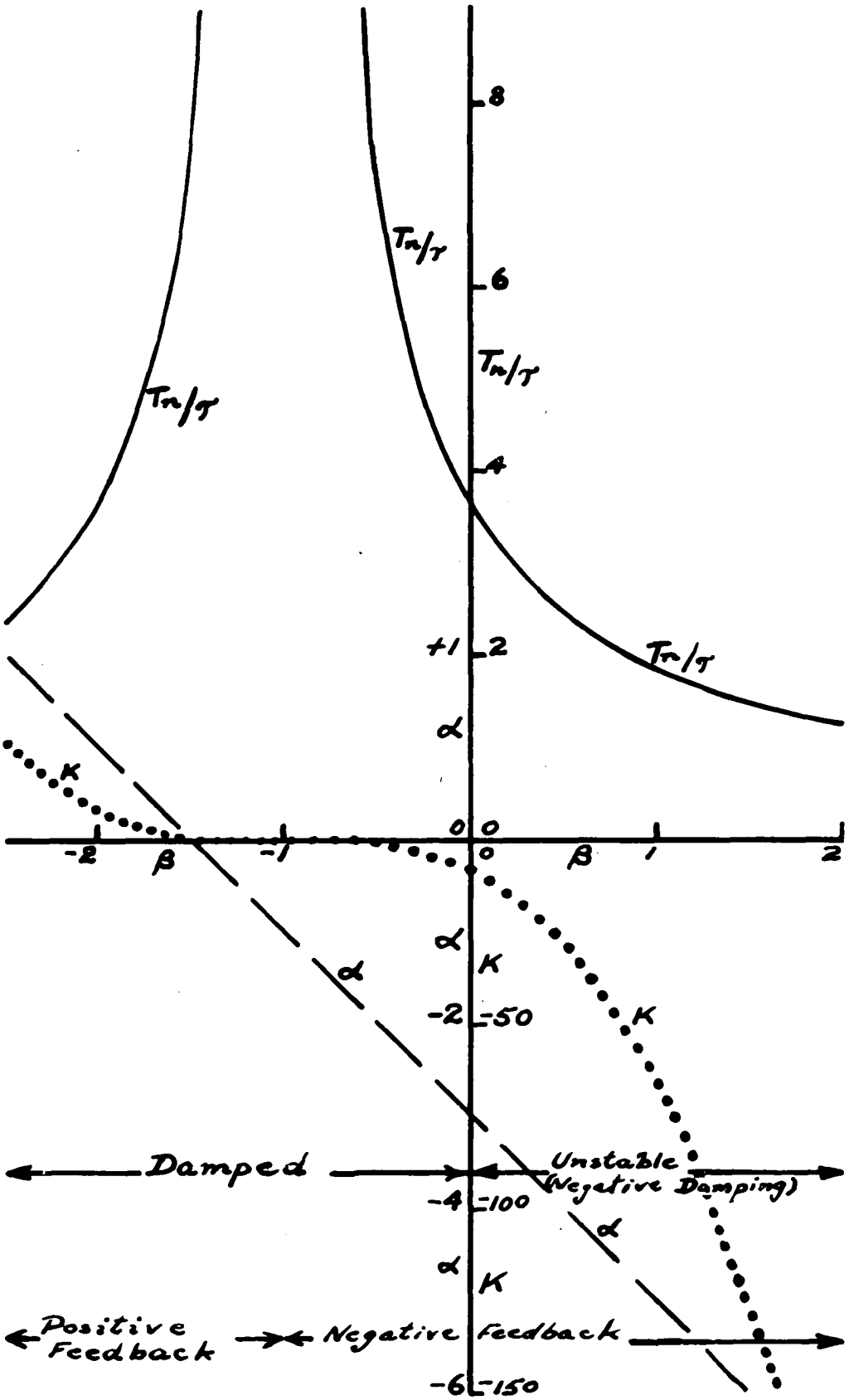
$$\gamma^2 + 2\alpha\beta = 3 \tag{21}$$

From these relations $(1 - K)$, T_n/τ and α may be plotted against β as shown in Figure 52.

An examination of Figure 52 reveals a considerable amount of information about the feedback loop. When β is positive the oscillations increase in amplitude with time (negative damping), that is, the system is unstable. When β is negative the oscillations are damped (positive logarithmic decrements). When α is positive, the loop has positive feedback while for negative values of α the feedback is negative and the loop acts as a control system for its variables, opposing any applied or endogenous disturbance. From equation (17) the time constant τ_e of the exponential component in the transient is given by

FIGURE 52

Calculated relations between the constants of equations (17) to (21).
The relation between α and β is given by the dashed line, that between K and β is dotted, while T_H/τ is denoted by the full curve.
The regions of positive and negative feedback are shown as well as those of stability (damped oscillations) and instability (negatively damped oscillations).


8
6
4
2
+1
0
0
-2
-1
0
1
2
β
β
α
K
-50
-100
-150
Tn/τ
Tn/τ
Tn/τ
Tn/τ
Damped
Unstable
(Negative Damping)
Positive
Feedback
Negative feedback

$$\tau_e = -\tau/\alpha$$

Similarly the time constant τ_v of the exponential envelope enclosing the damped oscillation is given by

$$\tau_v = -\tau/\beta$$

Hence $\tau = -\beta\tau_v$ (22a) or $\tau = -\alpha\tau_e$ (22b)

and

$$\beta/\alpha = \tau_e/\tau_v \qquad (23)$$

Consequently by measuring τ_e and τ_v from a transient the value of the ratio β/α may be determined from equation (23). The values of α and β may then be read off uniquely from Figure 52. Knowing the value of β, T_n/τ may also be read off from Figure 52. Let the value of T_n/τ be $\mathcal{K}$, that is

$$T_n/\tau = \mathcal{K} \qquad (24)$$

Combining equations (22a) and (24)

$$T_n = -\mathcal{K}\beta\tau_v \qquad (25)$$

Since $\mathcal{K}$ and β have both been determined, T_n may be calculated from equation (25).

By comparing this calculated value of T_n with the experimental value determined from the actual transient, the correspondence

between the actual system responsible for the transient and the assumed system may be tested.

3. Results

Transient potentials exhibiting damped oscillations of long periodicity such as those in Figure 9(b)(Section I,4) have been evoked by a considerable variety of different stimuli. These included the passage of electric current either alternating or direct, changing the plant's bathing solution from the culture medium (tap water) to the experimental bathing solution of 10^{-4}M KCl, exposure of the root to air and mechanical stimulation. Although the transients vary from plant to plant and depend on the type of stimulation, the same general features are present, namely the exponential component and the damped oscillations, sometimes very heavily damped.

Since the exponential component of the transient disappears within a few minutes, the oscillatory part can be studied separately after this time interval. By drawing the envelope of the oscillatory component, the time constant τ_v is determined. The oscillatory component is then extrapolated back to the time at which the transient began. By subtracting the oscillatory component from

the actual transient during the first few minutes, the exponential component is determined and its time constant τ_e is measured.

In Figure 53 the actual oscillatory periods T_a from potential transients are compared with the calculated period T_c. There is quite a good one to one correlation suggesting that the assumed feedback loop approximates reasonably well to the actual system producing the transients.

In Figure 54 the calculated values of τ are shown plotted against the actual periods of oscillation. A proportional relation exists between the two quantities. From the line of best fit for these points, the value of the ratio $T_n/\tau = 4.44$, which is in agreement with the predicted ratio for a three delay feedback system in which $\tau_1 = \tau_2 = \tau_3 = \tau$, as shown in Section III,8.

4. Discussion

Although the assumed feedback system fits the experimental data quite well, there may be many such systems which would fit the data equally well. By considering a number of other systems and determining how well these correlate with the case in which $\tau_1 = \tau_2 = \tau_3 = \tau$, some further information regarding the actual feedback system in the plant may be obtained.

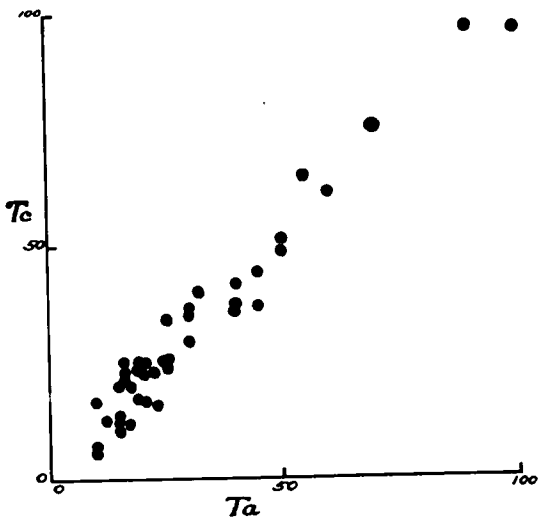
Figures 55(a) and (b) show curves similar to those in Figure 52

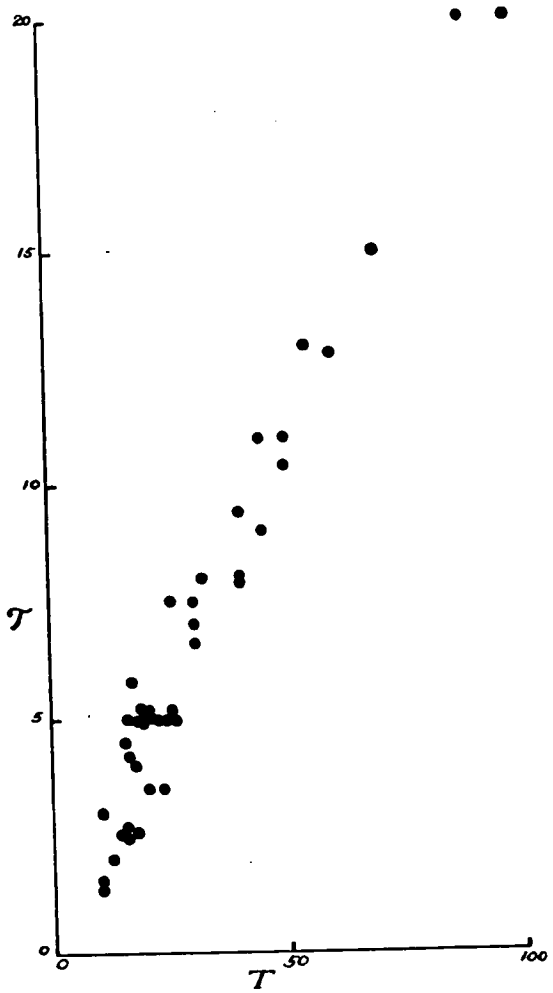
FIGURE 53

The correlation between the calculated oscillatory period (Tc) of the transient (Section V,2) and the actual value of the period (Ta) measured from the observed transient potential (Section V,3).

FIGURE 54

The correlation between the calculated value of the time delays (τ) ($\tau_1 = \tau_2 = \tau_3 = \tau$ assumed) in the feedback loop (Section V,2) and the period T measured from the observed transient potential (Section V,3).


100
50
0
Tc
0
50
100
Ta


20
15
10
5
0
T
0
50
100
T

relating $\mathcal{K}$ ($=T_n/\tau$), α and β for various sets of τ_1, τ_2, τ_3 values. In Figure 55(a) it is assumed that $\tau_1 = \tau_2 = \tau$. However τ_3 is allowed to take the values $\tau/10$, $\tau/2, \tau, 2\tau$ and 10τ. In Figure 55(b) the values τ_1, τ_2, τ_3 are taken as

$$\tau_1 = \tau, \quad \tau_2 = \tau, \quad \tau_3 = \tau$$

$$\tau_1 = \tau/2, \quad \tau_2 = \tau, \quad \tau_3 = 2\tau$$

$$\tau_1 = \tau/5, \quad \tau_2 = \tau, \quad \tau_3 = 5\tau$$

$$\tau_1 = \tau/10, \quad \tau_2 = \tau, \quad \tau_3 = 10\tau$$

From equation (25)

$$T_n/\tau_v = -\mathcal{K}\beta$$

Hence from the curves of Figure 55(a) and (b) relating $\mathcal{K}$, α and β for various sets of τ_1, τ_2, τ_3 the corresponding values of T_n/τ_v may be calculated for various τ_e/τ_v values. For various parametric values of τ_1, τ_2, τ_3, Figure 56 shows the comparison between the T_n/τ_v values and T_n/τ_v for $\tau_1 = \tau_2 = \tau_3$. The top scale shows the corresponding values of τ_e/τ_v for which the T_n/τ_v values were calculated.

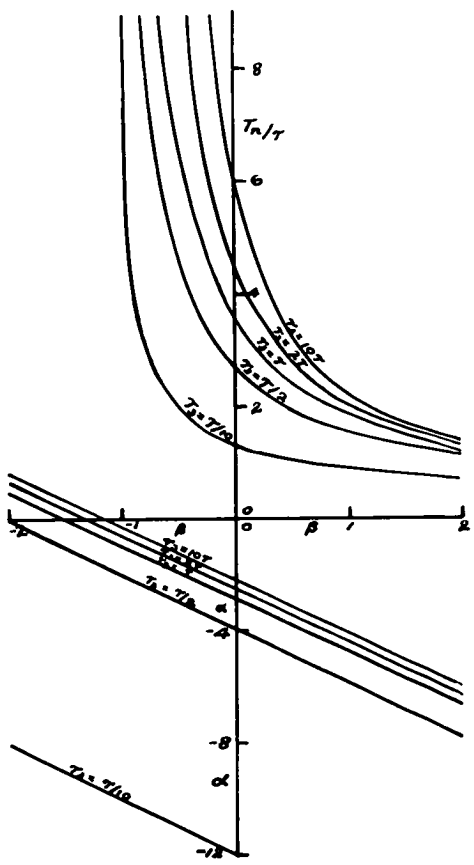
From Figure 56 it is apparent that for given experimental

FIGURE 55

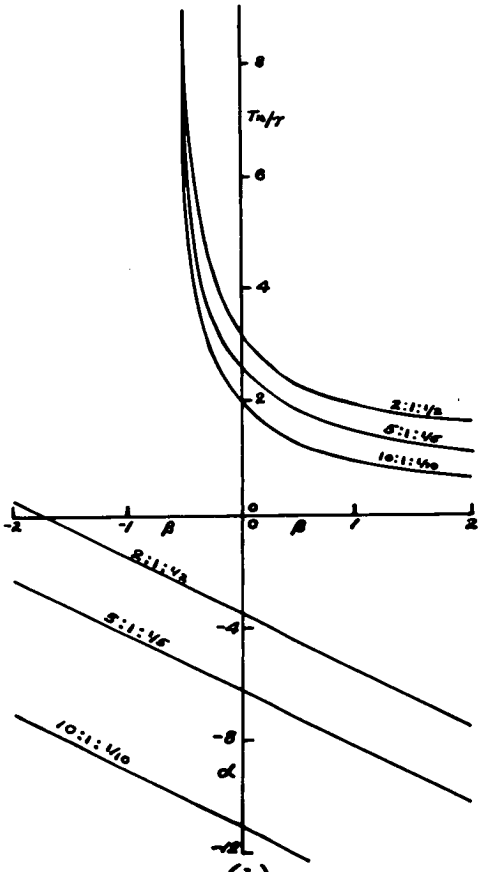
The calculated relations between α and β, Tn/τ and β for the cases in which

(a) $\tau_1 = \tau_2 = \tau$ and τ_3 takes values 10τ, 2τ, τ, $\tau/2$, $\tau/10$

(b)
$\tau_1 = \tau$, $\tau_2 = \tau$, $\tau_3 = \tau$
$\tau_1 = \tau/2$, $\tau_2 = \tau$, $\tau_3 = 2\tau$
$\tau_1 = \tau/5$, $\tau_2 = \tau$, $\tau_3 = 5\tau$
$\tau_1 = \tau/10$, $\tau_2 = \tau$, $\tau_3 = 10\tau$


8
T_n/T
6
4
2
$T_3=10T$
$T_3=5T$
$T_3=T$
$T_3=T/2$
$T_3=T/10$
-1
β
0
1
2
-2
-4
-8
α
-12

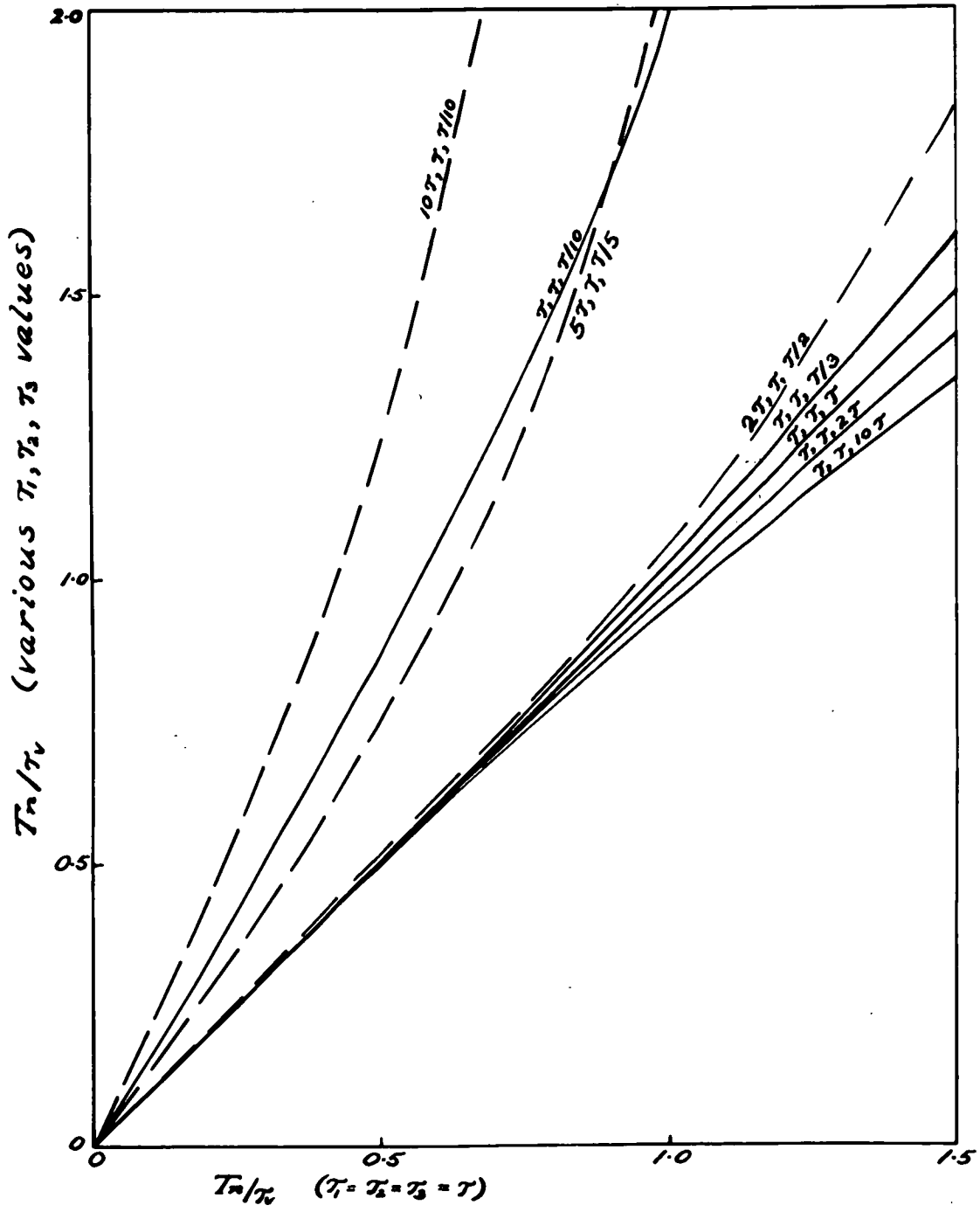
(a)


8
T_n/T
6
4
2
2:1:1/2
5:1:1/5
10:1:1/10
-1
β
0
1
2
-2
-4
-8
α
-12

(b)

FIGURE 56

The relation between T_n/τ_v calculated for various τ_1 , τ_2 , τ_3 values and T_n/τ_v calculated for $\tau_1 = \tau_2 = \tau_3 = \tau$.


2·0
1·5
1·0
0·5
0
0·5
1·0
1·5
T_n/T_v (various T_1, T_2, T_3 values)
T_n/T_v ($T_1 = T_2 = T_3 = T$)
10T, T, T/10
5T, T, T/5
T, T, T/10
2T, T, T/2
T, T, T/3
T, T, T
T, T, 2T
T, T, 10T

values of τ_e and τ_v, the calculated values of T_n/τ_v (and therefore of T_n) depend considerably on the assumed values of τ_1, τ_2, τ_3 in the three delay feedback loop. However there is a considerable range of τ_1, τ_2, τ_3 values over which the calculated values of T_n does not depart greatly from those calculated when τ_1, τ_2, τ_3 are assumed equal. For instance when τ_1 and τ_2 are equal ($=\tau$), τ_3 can take values ranging from 10τ to $\tau/3$ without substantially affecting the calculated values of T_n. If $\tau_3 = \tau/10$ however this is no longer true. Further if the ratio $\tau_1 : \tau_2 : \tau_3$ takes values $2:1:\frac{1}{2}$, $5:1:\frac{1}{5}$ or $10:1:\frac{1}{10}$ the calculated values of T_n depart very greatly from those when the three time delays are assumed equal.

It may be concluded that the experimental data obtained from the long period transient oscillations can be explained in terms of a three delay feedback loop. Further, the data is consistent with the situation in which the three delays are approximately equal, although a considerable range of other values of the three delays would fit the data equally well.

VI

THE RELATION BETWEEN THE APPLIED STIMULUS AND THE BIOELECTRIC RESPONSE

THE RELATION BETWEEN THE APPLIED STIMULUS AND THE BIOELECTRIC RESPONSE

1. Introduction

In previous sections the transient bioelectric potentials evoked by stimulation of the plant have been described without direct reference to the manner of stimulation. After removal of the stimulus it appears that the potentials recover to their previous steady values in accordance with a control mechanism in the plant not substantially dependent on the previous stimulation. Some correlation does exist however between the form of the transient potentials and the stimulus which evoked them. This is especially true with regard to the potential pattern around the root immediately following the removal of the stimulus.

In studying this correlation, electric stimulation has been used almost exclusively since it is difficult to control the degree of other types of stimuli in a strictly quantitative manner. It will be shown that the transient potential pattern of the root immediately after alternating voltage stimulation depends only on the number of cycles applied, irrespective of the frequency, provided that a sufficient quantity of electricity is passed through the plant during stimulation. The form of the relation is such that the bioelectric field of the plant root is disturbed less after

a large number of alternating voltage cycles than after relatively few cycles. This indicates that the plant adapts itself to the alternating voltage, the extent to which it does so being determined solely by the number of voltage cycles to which it has been subjected.

2. Experimental Methods

The plant under investigation was mounted vertically in the measuring tank with the root, but not the cotyledons, immersed in a bathing solution of 10^{-4} M KCl. The cotyledons in contact with moist cotton wool were held securely by an insulated stainless-steel clamp which acted also as one of the electrodes for the application of electric voltage stimulus, the other electrode being immersed a few centimetres below the root tip in the bathing solution.

While the external voltage was applied to the plant, the electrometer grid was earthed. On removal of the applied voltage the electrometer was switched in immediately to record the transient potentials of the plant root. Voltages, either direct or alternating, were applied between the two stainless-steel electrodes, a micro-ammeter being included in the circuit to record the total current passed through the plant.

Direct voltages were supplied by dry cells, the applied voltage stimulus being referred to as plus or minus according as the

cotyledons were made positive or negative with respect to the electrode in the bathing solution. Voltages of 9 and 18V, producing total currents through the plant of 100–250 μA, were employed. The duration of direct voltage application ranged from 0.01 to 300 sec. A relay circuit was employed to control the applications of short duration.

Alternating voltages of 10 and 20 V peak amplitude in the frequency range 0.1–10 c/s were supplied by a stable, low-frequency resistance-capacitance oscillator while a step-down transformer acting from the mains supply provided alternating voltages of frequency 50 c/s. Alternating voltages were applied for durations appropriate to provide numbers of cycles from 1 to 3000.

In order to assess the importance of the proximity of the bath electrode to the plant root and to determine which part of the root was most affected by the passage of current during electric stimulation, experiments were conducted to determine the distribution of applied current density flowing out from the plant root surface and into the bathing solution. This was done by exploring the region around the root with a measuring probe to determine the equipotential pattern. From this the current paths were determined and the current strengths passing out from various regions along the root were calculated.

It was found that the current density passing through the region of the root just below the bathing solution surface was about 100 times as great as that passing through the tip region, the current density falling off exponentially between the two regions. Further, the distribution of the applied current passing out from the root was found to be independent of the position of the bath electrode provided that it was situated more than 2 cm. below the root tip. In all experiments reported in this paper the separation between the root tip and the bath electrode was greater than this distance. The bathing solution could then be regarded as constituting the bath electrode.

General observations of growth rate and also microscopic examination of plant root sections revealed no injurious effects which might have been caused by the accumulation of ions during the passage of current within the range of currents and durations employed.

In some experiments the rate of elongation of the root was recorded. No significant changes were observed in the rate of elongation either during or following electric stimulation.

3. General Form of Transient Potentials

Following stimulation, the plant's potential pattern recovers eventually to a state not significantly different from that preceeding

stimulation, provided the stimulus is not too great. The form of the transient potential pattern depends on the particular type of stimulus applied and is independent of any previous electric stimuli which the plant has undergone provided sufficient time has been allowed for the potential pattern to recover to the normal unstimulated state.

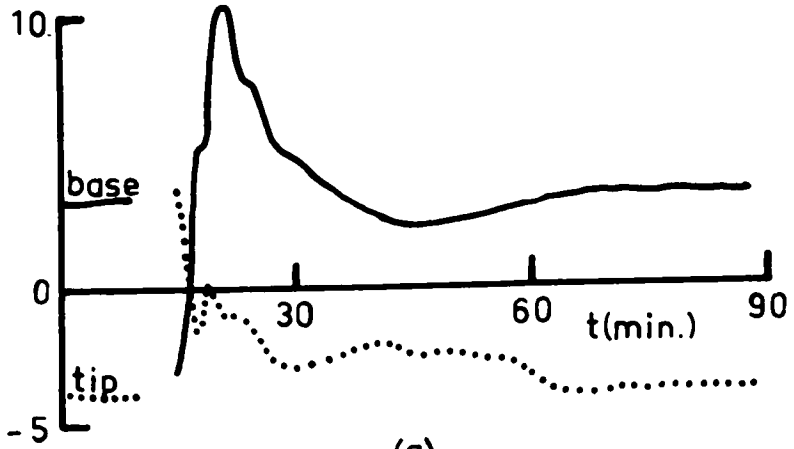
Figure 57 shows typical time courses of potentials near two representative points along the same plant root for three different successive electric stimuli. Figures 57(a) and (b) refer to direct voltage applications of opposite polarity while Figure 57(c) refers to alternating voltage application. Values of potentials prior to stimulation are shown at t = 0.

In Figures 57(a) and (b) it is apparent that the transients following the application of +10V for 300 sec. is different from those following -10V for the same duration. It is shown in Section VI, 5, that the direct voltage transients may be regarded as containing a component which is not dependent on the polarity of the applied voltage and a component which is.

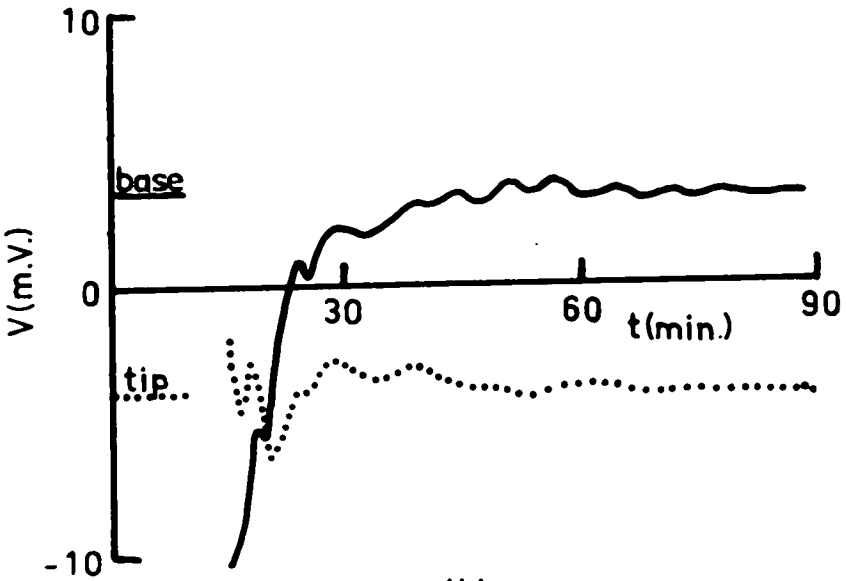
Since the transients resulting from alternating voltage stimulation are simpler in form than those for direct voltages, the results of the former will be described first.

FIGURE 57

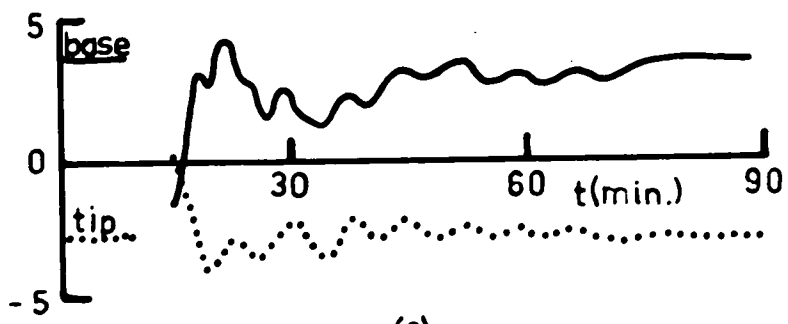
Time courses of transient potentials observed at the tip and basal regions of the same root. The stimuli applied are (a) +10 V, and (b) -10 V, and (c) 10 V peak alternating voltage, each applied for 300 sec. Steady potentials before stimulation are shown in each case, the gap in the record indicating the time of stimulation. For simplicity only two of the five transients normally recorded are shown. The three intermediate transients show a gradual transition in form between the two extremes shown.


10
base
0
30
60
t(min.)
90
tip
-5

(a)


10
base
V(m.V.)
0
30
60
t(min.)
90
tip
-10

(b)


5
base
0
30
60
t(min.)
90
tip
-5

(c)

4. Alternating Voltage Stimulation

In these experiments it was necessary to ascertain first that the resulting transient was not dependent on the phase in the cycle at which the alternating voltage stimulus was switched off. It might be reasonable to expect, for instance, that after applying one cycle of relatively long period, such as 10 sec, that the recovery transient would differ if the cycle were applied between two positive peaks (maxima), or between two negative peaks (minima). This was tested by averaging the appropriate transients obtained from 10 plants, each of which received a number of stimuli of the two extreme types cited. It was found that the corresponding pairs of average potential recovery curves did not differ significantly from one another. Hence it was concluded that transients caused by alternating voltage application are not dependent on the phase of the alternating cycle (or cycles) applied.

As shown in Figure 57(c), the characteristic feature of the alternating voltage transients is that the root tip potentials are algebraically increased while those of the root base are decreased immediately after stimulation, i.e. the electric polarity of the root tends to reverse. In general, the potential is decreased only at the root base, i.e. in the near vicinity of the bathing solution surface where most of the applied current flows through the

root surface, the rest of the potentials being increased, though often to a lesser extent.

It is evident that an index of the effect caused by stimulation is given by the deviation in plant potential (at a particular point along the root) immediately after removal of the stimulus. This deviation (D) with sign attached, may then be used to compare the effects caused by different electric stimuli.

In Figure 58, D (at the root base) is plotted against the frequency of the alternating voltage applied for a constant duration of 300 sec, while, in Figure 59, D is plotted against duration of application at a constant frequency of 1 c/s. In both cases the peak voltage was 10V throughout.

It is seen that the magnitude of D decreases with both increasing frequency and duration of application. This suggests that the magnitude of D decreases with the number of cycles of alternating voltage applied. In Figure 60, D is plotted against the number of cycles, employing frequencies of 50, 10, 1, and 0.1 c/s, applied for appropriate durations.

It appears that D depends uniquely on the number of cycles applied at a constant peak voltage, except for the cases in which the stimulus is applied for durations less than about 1 sec (viz. 50 c/s for 1-30 cycles, and 10 c/s for 1-10 cycles).

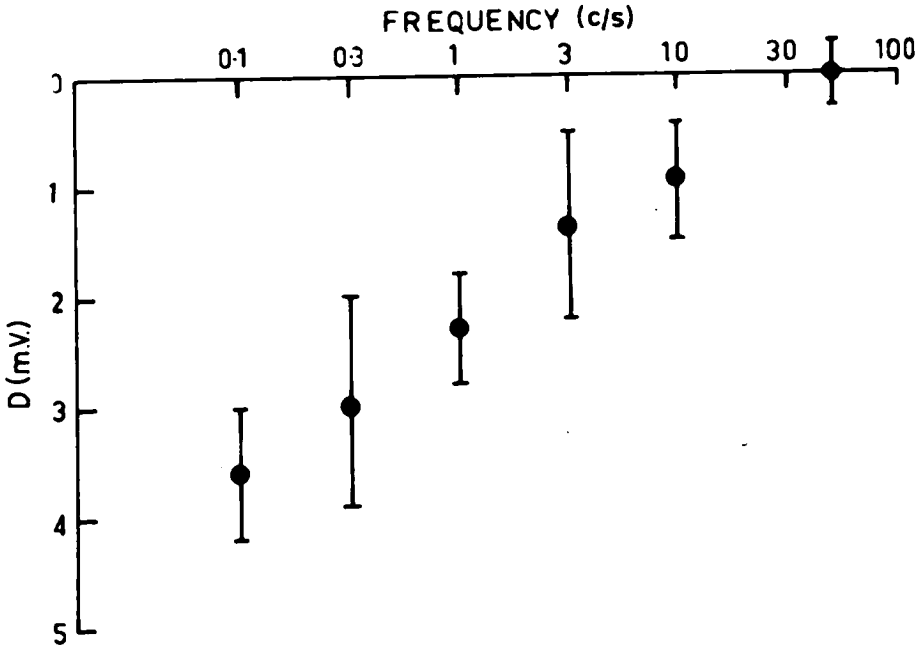
The fact that for very brief applications of the higher frequency

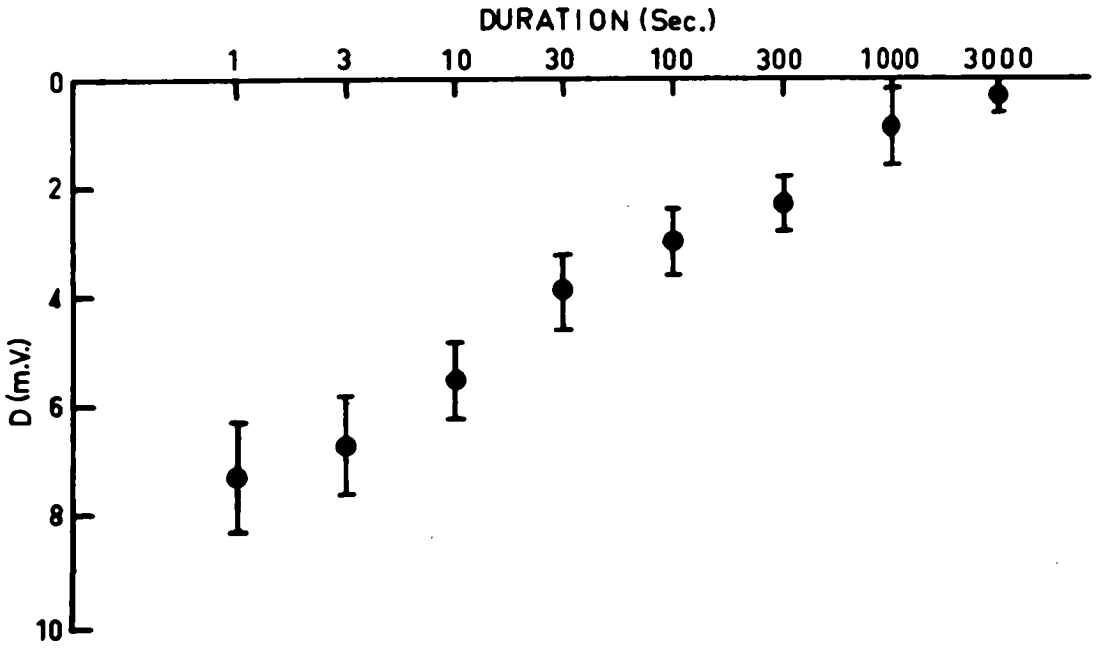
FIGURE 58

Relations between D (at the root base) and the frequency of the 10V peak voltage applied for 300 sec. Ninety-fivepercent confidence limits are indicated (10–20 plants).

FIGURE 59

Relation between D (at the root base) and the duration of the applied 10V stimulus at 1 c/s. Ninety-five per cent confidence limits are indicated (20 plants).


FREQUENCY (c/s)
0·1
0·3
1
3
10
30
100
0
1
2
3
4
5
D (m.V.)


DURATION (Sec.)
1
3
10
30
100
300
1000
3000
0
2
4
6
8
10
D (m.V.)

alternating voltages, the curve departs from the unique relation between D and the number of cycles, implies that insufficient electricity is passed through the plant during such short applications. It is possible to pass this required amount of electricity through the plant by applying a single initial cycle of 1 sec. period. If this is done and then followed immediately by a number of cycles of frequency 50 c/s, the relation between D and the number of cycles then becomes identical with that for lower frequencies such as 1 and 0.1 c/s (Figure 60) even if the number of cycles is small.

This is shown in Figure 61 in which the 10V 50 c/s curve from Figure 60 is redrawn. The other 10V curve (open circles) shows 10V 50 c/s data obtained after first applying an initial cycle of 1 sec. period. It is apparent that this curve is identical with that in Figure 60 for the 1 c/s and 0.1 c/s points.

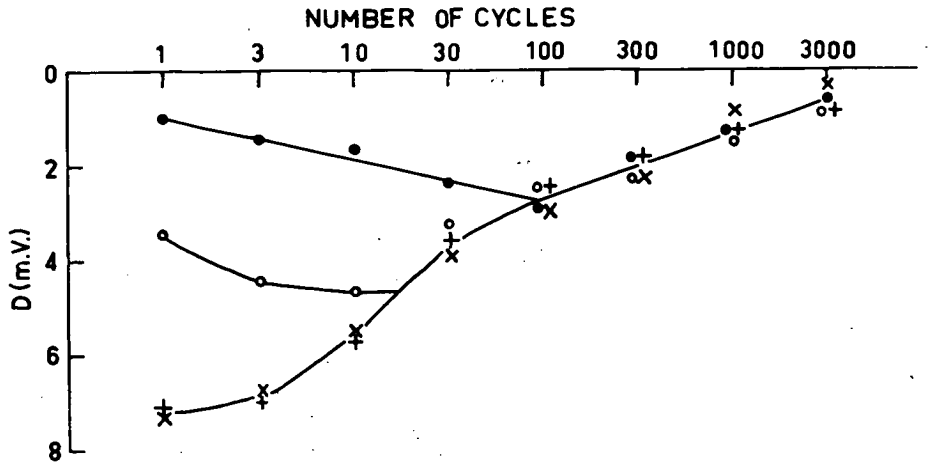
Data obtained similarly for 20V 50 c/s is shown in Figure 61 also. The relations between D and the number of cycles at 20V are obviously similar to those at 10V but the magnitude of D is greater. The point at which the upper and lower constant voltage curves join indicates the number of cycles for which just sufficient charge has been passed through the plant to make the relation between D and the number of cycles independent of frequency. For 50 c/s

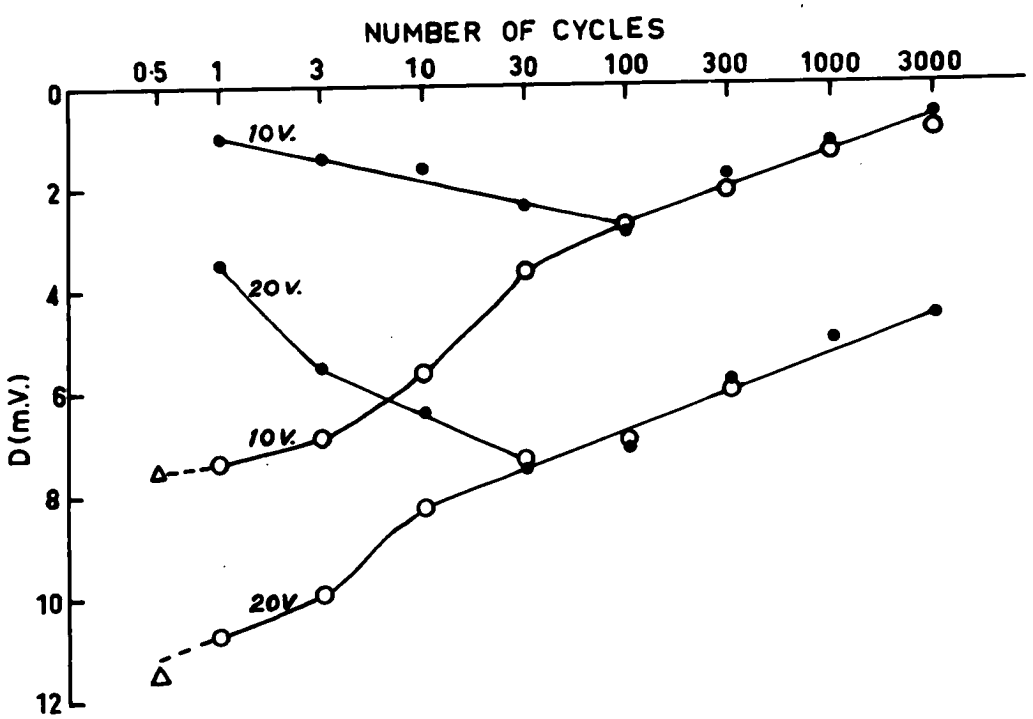
FIGURE 60

Relation between D(at the root base) and the number of cycles of the applied 10V peak voltage at frequencies of 50; 10; 1 and 0.1 c/s. Ninety-five per cent confidence limits are similar in magnitude to those in Figure 3 (20 plants).

FIGURE 61

Relations between D (at the root base) and the number of cycles at 50 c/s(●) of 10V (upper curve) and 20V (lower curve). ○ Values of D obtained when a single cycle of period 1 sec. is applied immediately before the 50 c/s voltage application. Ninety-five percent confidence limits for all points are similar to those in Figure 3 (15 plants). Points at 0.5 cycles (Δ) are referred to in Section IV.


NUMBER OF CYCLES
1
3
10
30
100
300
1000
3000
0
2
4
6
8
D(m.V.)


NUMBER OF CYCLES
0·5
1
3
10
30
100
300
1000
3000
0
2
4
6
8
10
12
D(m.V.)
10V.
20V.
10V.
20V

at 10V this point is at 100 cycles, while for 20V, only 30 cycles is sufficient.

This suggests that the passage of a constant quantity of charge is necessary before the dependence of D on the number of cycles becomes independent of the frequency. As is shown below, it appears that this required quantity of charge is independent of the applied voltage and its frequency.

Values of this quantity of charge Q have been determined from measurements of the average value of alternating current passed through plants during stimulation. Q is then given by the product of the average current and the necessary duration for the dependence of D on the number of cycles to become independent of frequency. Three cases have yielded the following average values of Q (Table 2). Twenty plants were used in each case. Considering the logarithmic type of relation between D and the number of cycles, there is quite good agreement between the values of Q obtained.

The form of the relations between D and the number of cycles at other points along the root is identical with those described for the basal regions (i.e. at points on the root just below the surface of the bathing solution). However, as mentioned previously in this section, D changes sign and decreases in magnitude a few millimetres below the bathing solution surface.

TABLE 2

INDEPENDENCE OF QUANTITY OF CHARGE ON APPLIED VOLTAGE AND FREQUENCY

Each result is the mean of 20 plants

Voltage (V)	Frequency (c/s)	Q (coulombs)
10	10	$9 \pm 5 \times 10^{-5}$
10	50	$14 \pm 5 \times 10^{-5}$
20	50	$10 \pm 5 \times 10^{-5}$

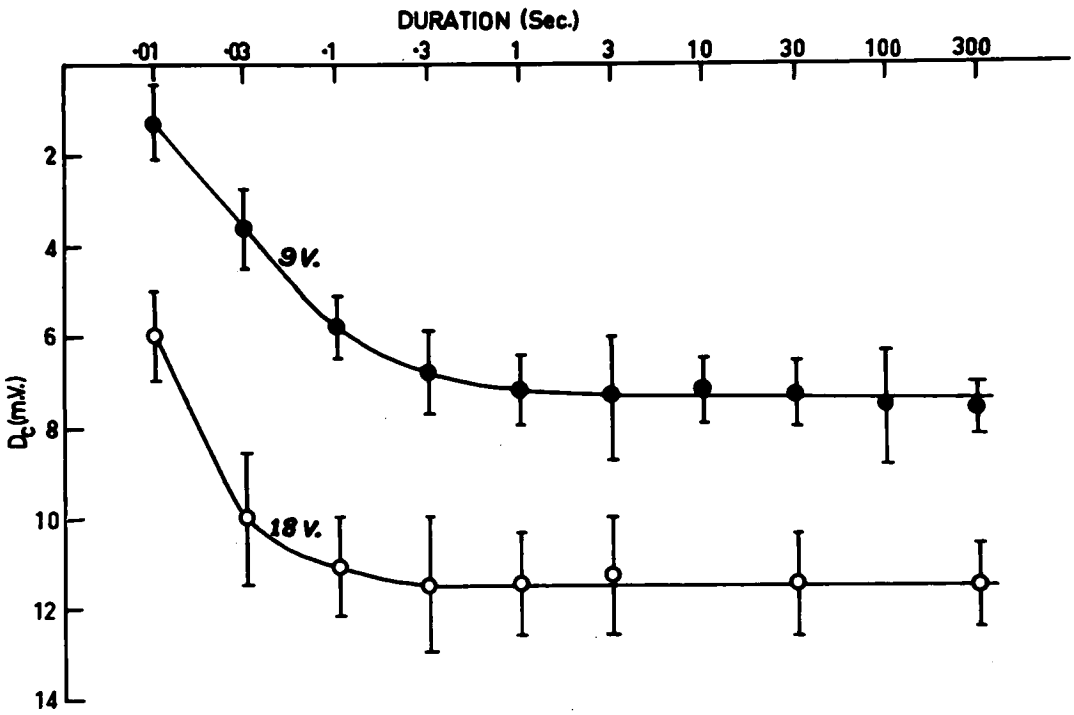
5. Direct Voltage Stimulation

From sets of transients such as those of Figures 57(a) and (b) obtained from a number of plants, it is found that the average recovery curves of potential are significantly different depending on whether the direct voltage is applied in a positive or in a negative sense with respect to the bathing solution. However, the averages of corresponding pairs of transients induced by equal but opposite direct voltage stimuli in a number of plants are found to be very similar to those obtained from alternating voltage stimulation. These average curves will be referred to as the "common" components of the direct voltage transients and may be regarded as their components not dependent on the sign of the applied voltages. Only the common components will be discussed in this paper. As in the alternating voltage results, the characteristic feature of the common components is that the potentials of the very basal end of the root are decreased while those of the regions nearer the tip are algebraically increased immediately after removal of the applied direct voltage. Again, an index of the common effect may be defined as the initial deviation, D_c, following stimulation of the common potential curve from the unstimulated plant's potential.

Figure 62 shows D_c (at the base) plotted against duration of application of both 9 and 18V. These graphs show that the

FIGURE 62

Relations between D_c (at the root base) and the duration of application of 9 and 18 V. Ninety-five percent confidence limits are indicated (20 plants).


DURATION (Sec.)
·01
·03
·1
·3
1
3
10
30
100
300
2
4
6
8
10
12
14
D_c (m.V.)
9 V.
18 V.

magnitude of D_c increases with duration up to about 1 sec. for 9V and about 0.3 sec. for 18V after which D_c is independent of the duration of voltage application but its magnitude increases with voltage.

This again indicates a charge requirement before D_c becomes independent of duration, the average value of this charge Q_c for the two cases (9 and 18V) being 9×10^{-5} coulombs. This may be compared with the average value of $Q = 11 \times 10^{-5}$ coulombs, obtained in the alternating voltage case (Section VI, 4).

6. Discussion

In Section VI, 4, it was seen that D depends uniquely on the number of cycles of constant peak voltage applied, provided that the amount of charge passed through the plant during stimulation exceeds a definite quantity $Q(=11 \times 10^{-5}$ coulombs approximately). This quantity of charge would be transported through the plant by 11.4×10^{-10} g-equiv. of ions. It was shown further (Figure 61) that after this amount of charge has been passed by applying a single cycle of period 1 sec, the relation between D and the number of cycles at 50 c/s applied subsequently is identical with the unique relation between D and the number of cycles applied at lower frequencies such as 1 and 0.1 c/s, at which frequencies the

required amount of charge is passed during only one cycle.

The relation between D_c and duration of 9- and 18-V pulses was shown to exhibit a similar effect in that the magnitude of D_c increases with duration until a charge of Q_c ($=9 \times 10^{-5}$ coulombs) has been passed, after which D_c remains constant (Figure 62).

A single direct voltage pulse of about 10-V amplitude might be interpreted as half a cycle of 10-V peak alternating voltage. In this case it would be expected that D_c should be independent of the pulse length (provided it is 1 sec. or longer) since the number of cycles (viz. a half cycle) remains the same whatever the pulse length is. This may be compared with the alternating voltage case in which single cycles of period 1 sec. or longer were seen to cause identical values of D.

Figure 61 shows the 10- and 20-V curves relating D at the base and the number of cycles. The points shown at 0.5 cycle indicate the values of D_c for 9- and 18-V pulses of duration greater than 1 sec. It is apparent that these points are close to the extrapolations (dotted) of the 10- and 20-V alternating voltage curves respectively.

This, together with the fact that the values of Q($=11 \times 10^{-5}$ coulombs) and Q_c ($=9 \times 10^{-5}$ coulombs) are in agreement, suggests

that the direct voltage common effect and the alternating voltage effect are identical.

Having combined the two effects in this way, the phenomenon may be summarised as follows. The transient potentials observed immediately after the removal of an electric stimulus depend on two factors: the total quantity of charge passed through the plant, and the number of alternations of the applied voltage. As the quantity of charge passed through the plant is increased the magnitude of the transients (measured by D or D_c) increases until its maximum value is reached with the passage of approximately 10^{-4} coulombs. This quantity of charge and the value of D or D_c is independent of the manner in which the charge is passed although the value of D or D_c increases with the magnitude of the applied voltage.

In addition to this effect there is that of the number of cycles in which the magnitude of D decreases approximately logarithmically with the number of cycles applied.

Marsh (1930) showed that following the passage of direct current along onion (Allium cepa) roots, the potential changed and eventually returned to the unstimulated state. The potential change was found to increase with the amount of charge passed along the root while the polarity of the potential change depended solely on

the direction in which the current flowed, i.e. no common change was observed even though the quantities of charge passed through the roots ranged from 10^{-5} coulombs to values as large as 10^{-3} coulombs. However, these experiments were conducted in a rather different manner from those described in this paper. The electrodes, used both for measuring root potentials and for the application of electric current, consisted of ring electrodes containing tap water placed at various positions along the root growing in air saturated with water vapour.

It is to be expected that both the stimulated and the unstimulated root's potential pattern should differ for the two methods of measurement, since potentials, either steady or transient, produced in a bathing solution depend on the flow of bioelectric current through the solution about the root (Scott, McAulay and Jeyes 1955), whereas for a root in air, a more static potential is measured.

Danisch (1921) showed that Vorticella nebulifera adapts itself to the application of a succession of mechanical shocks of known energy. For instance, after nine impulses, each of 500 ergs, the organism no longer responds to repeated impulses, whereas for 2000-erg impulses, 420 are required. Danisch concluded that the effect was one of habituation rather than fatigue. These results appear to be similar to those described in this paper.

A possible interpretation of the phenomenon may be sought by considering the electrical configuration of fixed ions in the tissue to which mobile ions are loosely bound. The fixed ions could be situated in membranes or in the cytoplasm, i.e. the large immobile anions of the Donnan phase. Some of the ionic bonds are stronger than others so that the application of an electric field would be expected to break the weaker bonds. The number of bonds broken would depend on the electric field strength and not on the duration of the application, provided it is applied sufficiently long to allow the mobile ions to be stripped from their sites of binding.

Having broken these ionic bonds, the mobile and immobile ions separate under the influence of the field. This change in the electrical configuration would alter the bioelectric field especially if the site of ionic separation were at the cell membranes. If the applied field then reverses successively, as in the application of alternating voltage, the separated ions of opposite sign would be moved back and forth under the influence of the oscillating electric field. This would allow the ions of opposite polarity to recombine in more closely bound configurations where the binding is sufficiently strong to be unaffected by the applied field. Thus the final state after the rearrangement produced by a large number of electric field reversals would be a more closely bound form

of the original electrical configuration. That is, the final electrical state and hence the bioelectric field would be substantially the same as before the electric field application. In this way the plant becomes "adapted" to the application of a large number of alternating voltage cycles so as to show a diminished change in the bioelectric field compared with that resulting from the application of a small number of cycles or a direct voltage.

This type of adaptability is essentially a stochastic control process. An initial change in environment (the application of an electric field) leads to a sudden change in the physiological system, manifested in the bioelectric potential pattern. Subsequent reversals of the initial environmental change however allow the system to return to a configuration similar to that originally obtaining but which is more stable.

Although this process has been described in terms of fixed and mobile ions there are probably many other aspects of the physiological system which could be affected in a similar manner. For instance, polar molecules such as proteins in the membranes of plant cells are more or less loosely bound to one another in the normal physiological state. The application of an electric field would break these loose bonds and cause alignment of the polar molecules again leading to an alteration of the electrical configuration.

In a manner similar to that described previously, reversals of the electric field would force the polar molecules to swing back and forth so that the polar ends of different molecules would be brought into close proximity so as to unite and eventually form closed chains or domains of molecules of a more closely bound configuration than originally.

VII

FINAL DISCUSSION AND CONCLUSIONS

VII

FINAL DISCUSSION AND CONCLUSIONS

In this thesis it has been shown that the effect of stimulation, in evoking a change in the bioelectric potentials of bean root tissue, appears to be distinct from the recovery process of the potentials to the normal resting state following the removal of stimuli. The latter recovery process appears to obey the principles of a negative feedback control system acting within the plant. The initial stimulus merely changes the values of the physiological variables in the feedback loop to values different from those obtaining in the unstimulated plant, maintained in an unchanging environment.

It was shown however that a control mechanism exists in the plant even while electric stimuli are being applied (Section VI). Attempts to explain this control system which is dependent on the number of cycles of alternating current applied, in terms of a feedback system proved unsuccessful. Consequently the stochastic control system described in Section VI was postulated. It is to be expected that the control system of Section VI applicable even for short time periods (1 second) and at high frequencies (up to at least 50 c/s) relative to those involved in the feedback control

systems of Sections III and V, would be fundamentally different from such negative feedback systems.

Evidence of the feedback control systems arose from the observation of bioelectric oscillations. As yet the spontaneous oscillations, which occur infrequently, appear to be only a symptom of instability in the negative feedback loop of control for the physiological variables involved. There is as yet no evidence to suggest that the spontaneous potential oscillations play any important role in the physiological system such as that of the potential oscillations associated with aggregates of neurones in the animal nervous system. It appears rather that aggregates of cells in a wide diversity of living tissues are inherently capable of oscillatory potential behaviour as the result of some unstable feedback system involved in the tissues. In the central nervous system however this fundamental property is used to render the neurones more sensitive to small stimuli so that the full potential response, namely action potentiation, is more readily evoked.

It is possible that when in the oscillatory state, the plant's physiological system is rendered more sensitive to environmental changes so that the system may adjust itself more readily to oppose any divergence from optimum conditions of the system. For example, a plant root in the oscillatory condition (unstable

feedback system) may be more sensitive to changes in the direction of the gravitational field (e.g. if the root be rotated from the vertical position). In this way the plant root would respond more readily and bend more quickly towards the vertical position again.

It may be concluded that the study of potential oscillations in bean roots has given a new insight into the complex and subtle interaction of physiological variables and the way in which this interaction controls them in the biological organ. The methods and concepts of feedback system analysis appear to offer a valuable tool in the study of physiological systems generally.

VIII

REFERENCES

REFERENCES

ABERG, B. (1957) - Ann. Rev. Plant Physiol. 8 : 153.

ADAMSON, D. and ADAMSON, Heather (1958) - Science 128 : 532.

ADOLPH, A. R. (1959) - Bulletin Math. Biophys. 21 : 195.

AMLONG, H. U. (1933) - Planta 21 : 211.

ARVANITAKI, A. (1943) - Arch. Internat. Physiol. 53 : 508.

BACKUS, G. E. and SCHRANK, A. R. (1952) - Plant Physiol. 27 : 251.

BENNET-CLARK, T. A. (1955) - "The Chemistry and Mode of Action of Plant Growth Substances "(ed. Wain, R. L. and Wightman, F. Butterworths, London) 284.

BRAUNER, L. and BUNNING, E. (1930) - Ber. d. Bot. Geo. 48 : 470.

BRIGGS, W. R., and TOCHER, R. D., and WILSON, J. F. (1957) - Science 126 : 210.

BROWN, R. and SUTCLIFFE, J. F. (1950) - J. Exp. Bot. 1 : 88.

BURSTROM, H. (1950) - Physiol. Plant. 3 : 277.

BURSTROM, H. (1951a) - Physiol. Plant. 4 : 199.

BURSTROM, H. (1951b) - Physiol. Plant. 4 : 407.

CLARK, W. G. (1937) - Plant. Physiol. 12 : 737.

COLE, K. S. (1941) - J. Gen. Physiol. 25 : 29.

COOMBS, J. S., ECCLES, J. C. and FATT, P. (1955) - J. Physiol. 130 : 374.

DANISCH, F. (1921) - Z. Allg. Physiol. 19 : 133.

ECCLES, J. C. (1953) - "The Neurophysiological Basis of Mind : The Principles of Neurophysiology" (Clarenden Press, Oxford) 193 and 228.

ECCLES, J. C. and JAEGER, J. C. (1958) - Proc. Roy. Soc. B 148 :38-56.

FINDLAY, G. P. (1959) - Aust. J. Biol. Sci. 12 : 412.

FREY-WYSSLING, A. (1949) - Exp. Cell. Research. suppl. 1 : 33.

FREY-WYSSLING, A. (1953) - "Submicroscopic Morphology of Protoplasm and its Derivatives". (Elsevier Publishing Co., Amsterdam).

FREY-WYSSLING, A. (1955) - Protoplasmatologia 2, pt. 2 : 189.

GOLDACRE, R. J. (1952) - Int. Rev. Cytol. 1 : 135.

GOLDACRE, R. J. and LOCH, I. J. (1950) - Nature 166 : 497.

HAY, J. R. (1931) - Pl. Physiol. 31 : 118.

HODGKIN, A. L. and HUXLEY, A. F. (1952) - J. Physiol. 117 : 500.

JENKINSON, I. S. (1958) - Biol. Sci. 11 : 485.

KISCHIMOTO, Uichiro (1958) - J. Gen. Physiol. 41 : 1205.

KISCHIMOTO, Uichiro (1958) - J. Gen. Physiol. 41 : 1223.

KOCH, K. (1934) - Planta 22 : 190-220.

LING, G. and GERARD, R. W. (1949) - J. Cell. and Comp. Physiol. 34 : 413.

LOEWY, A. G. (1949) - Proc. Am. Phil. Soc. 93 : 326.

LOEWY, A. G. (1950) - J. Cell. and Comp. Physiol. 35 : 151.

LOEWY, A. G. (1952) - J. Cell. and Comp. Physiol. 40 : 127.

McAULAY, A. L. and SCOTT, B. I. H. (1954) - Nature 174 : 924.

McRAE, D. H. and BONNER, J. (1953) - Physiol. Plant. 6 : 485.

MARSH, G. (1930) - Protoplasma 11 : 447.

MERCER, F. V., HODGE, A. J., HOPE, A. B. and McLEAN, J. D. (1955) - Aust. J. Biol. Sci. 8 : 1.

MUELLER, Paul (1958) - J. Gen. Physiol. 42 : 39.

MUELLER, Paul (1958) - J. Gen. Physiol. 42 : 163.

MUELLER, Paul (1958) - J. Gen. Physiol. 42 : 193.

ODA, K. (1956) - Sci. Rep. Tohoku Univ. (4) 22 : 167.

OSTERHOUT, W. J. V. (1931) - Biol. Rev. 6 : 369.

OSTERHOUT, W. J. V. (1934) - J. Gen. Physiol. 18 : 215.

OSTERHOUT, W. J. V. (1936) - Physiol. Rev. 16 : 216.

PITTENDRIGH, C. S. and BRUCE, V. G. (1957) - "Rhythmic and Synthetic Processes in Growth" (Ed. Dorothea Rudnick, Princeton University Press) : 75.

SACHER, J. A. (1957) - Science 125 : 1199.

SACHER, J. A. (1959) - Plant. Physiol. 34 : 365.

SACHER, J. A. and GLASZIOU, K. T. (1959) - Nature 183 : 757.

SCOTT, B. I. H. (1957) - Aust. J. Biol. Sci. 10 : 164.

SCOTT, B. I. H., McAULAY, A. L., and JEYES, P. (1955) - Aust. J. Biol. Sci. 8 : 36.

SCHRANK, A. R. (1945) - Plant. Physiol. 20 : 133.

SCHRANK, A. R. (1945) - Plant. Physiol. 20 : 344.

SCHRANK, A. R. (1946) - Plant. Physiol. 21 : 362.

SCHRANK, A. R. (1946) - Plant. Physiol. 21 : 967.

SCHRANK, A. R. and BACKUS, G. E. (1951) - J. Cell. and Comp. Physiol. 38 : 361.

SCHRANK, A. R. (1951) - "Plant Growth Substances" (ed. Folke Skoog, Univ. of Wisconsin Press) : 123.

SCHRANK, A. R. (1957) - "Symposia of the Society for Experimental Biology No. XI - The Biological Action of Growth Substances" : 95.

SEIFRIZ, W. (1942) - "Structure of Protoplasm" (W. Seifriz Ed.) (The Iowa State College Press, Ames) : 245.

SEIFRIZ, W. (1943) - Bot. Rev. 9 : 50.

SJODIN, R. A. and MULLINS, L. J. (1958) - J. Gen. Physiol. 42 : 39.

STARK, L., CAMPBELL, F. W. and ATWOOD, J. (1958) - Nature 182 : 857.

STARK, L. and CORNSWEET, T. N. (1958) - Science 127 : 588.

TAGAWA, T. and BONNER, J. (1957) - Plant. Physiol. 32 : 207.

TEORELL, Torsten (1959a) - J. Gen. Physiol. 42 : 831.

TEORELL, Torsten (1959b) - J. Gen. Physiol. 42 : 847.

VAN OVERBEEK, J. (1959) - Bot. Rev. 25 : 269.

WALKER, N. A. (1955) - Aust. J. Biol. Sci. 8 : 476.

WEBSTER, W. W. Jr. and SCHRANK, A. R. (1953) - Arch. Biochem. and Biophys. 47 : 107.

WIEGAND, O. F. and SCHRANK, A. R. (1954) - Arch. Biochem. and Biophys. 56 : 459.